Atty. Docket: UCONBA/186/US

In re patent application of Ben A. Bahr

Application No.:	10/056,666	Examiner:	M.V. Meller
Filing Date:	October 29, 2001	Group Art Unit:	1654

For: Materials For Lysosome Modulation and Methods of Use Thereof

TRANSMITTAL LETTER

Mail Stop Appeal Brief – Patents Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450
Attention: Board of Patent Appeals and Interferences

Sir, enclosed herewith is (are):

- Appeal Brief
- Check in the amount of $ 250.00
- return postcard

It is hereby petitioned that any required extension of time be granted for filing the enclosed papers. An extension of 0 month(s) having a fee of $0 appears required.

☐ If checked, claims are being amended.

	No. of claims remaining after amendment	Highest no. of claims previously paid for	no. extra	claim fee	subtotal
Total				$	$0.00
Independent				$	$0.00
			Total claim fees		$ 0.00

☒ If checked, a check in the amount of $ 250.00 is attached. Please credit any overpayment to Deposit Account 16-2563 of Alix, Yale & Ristas, LLP.

The Commissioner is hereby requested and authorized to charge Deposit Account 16-2563 of Alix, Yale & Ristas, LLP for any required extension fee and for any other fee, not enclosed herewith, due for any reason during the pendency of this application or in connection with the accompanying document, including (a) any filing fees under 37 CFR 1.16 for the presentation of extra claims and (b) any patent application processing fees under 37 CFR 1.17. A duplicate copy of this letter is enclosed.

Date: 4/4/05

By: _____
Thomas A. Hatfield
Registration No. 47,637
Attorney for Applicant

Alix, Yale & Ristas, LLP
750 Main Street- Suite 1400
Hartford, CT 06103-2721
(860) 527-9211

EXPRESS MAIL Mailing Label Number: EV 429950612 US

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" Service under 37 CFR 1.10 on April 4, 2005, and is addressed to the "Mail Stop Appeal Brief – Patents Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, Attention: Board of Patent Appeals and Interferences".

_____
James E. Piotrowski

G:\AYR saved docs\Filing Docs\Uconba\4-4-05 transmittal.doc

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of:

Ben A. Bahr

Application No.:	10/056,666	Examiner:	M.V. Meller
Filing Date:	October 29, 2001	Group Art Unit:	1654

For: Materials For Lysosome Modulation and Methods of Use Thereof

To: Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

ATTENTION: Board of Patent Appeals and Interferences

Honorable Judges:

APPEAL BRIEF

APPEAL BRIEF

This brief contains the following sections under the headings and in the order set forth below as required by 37 C.F.R. §41.37.

I. **REAL PARTY IN INTEREST** (37 C.F.R. §41.37(c)(1)(i))

II. **RELATED APPEALS AND INTERFERENCES** (37 C.F.R. §41.37(c)(1)(ii))

III. **STATUS OF CLAIMS** (37 C.F.R. §41.37(c)(1)(iii))

IV. **STATUS OF AMENDMENTS** (37 C.F.R. §41.37(c)(1)(iv))

V. **SUMMARY OF CLAIMED SUBJECT MATTER** (37 C.F.R. §41.37(c)(1)(v))

VI. **GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL** (37 C.F.R. §41.37(c)(1)(vi))

VII. **ARGUMENT** (37 C.F.R. §41.37(c)(1)(v1i))

A. **New Claim 25 Included in Appellant's Rule 116 Amendment Filed on December 29, 2004 Should be Entered and Considered in this Appeal.**
   1. **The amendment in dispute.**
   2. **Requirements for the entry of amended claims Under 37 C.F.R. §1.116.**
   3. **The record concerning the disputed claims.**
   4. **The Board should enter and consider Appellant's Rule 116 Amendment as filed on December 29, 2004.**

B. **Appellant's Claims 9-12, 14, and 24 are Enabled Under 35 U.S.C. § 112, First Paragraph and Therefore Patentable.**
   1. **The legal burden necessary to properly assert a 35 U.S.C. §112, first paragraph rejection.**
   2. **The Asserted Rejections Under 35 U.S.C. §112, First Paragraph.**
   3. **The Office communication assertions do not meet the required legal burden to assert a 35 U.S.C. §112, first paragraph, rejection.**
      a. **The Office communication assertions are contrary to knowledge in the art.**
      b. **Appellant's claims reciting treatment for Alzheimer's disease are enabled.**
      c. **Appellant's claims reciting treatment for Parkinson's disease are enabled.**
      d. **Appellant's claims reciting treatment for Lysosomal Storage Disorder are enabled.**
      e. **Appellant's claims reciting treatment for Lysosomal Storage**

Disorder are enabled based on FDA approved labels.

f. Appellant's claims reciting treatment for Lysosomal Storage Disorder are enabled based on recent scientific investigation.

4. The Rejections of Claims 9-12, 14, and 24 are Clearly Arbitrary Based on the Allowance of Claim 13

5. The Office Communication of February 1, 2005 is contrary on its face to the development of the issues with respect to the asserted 35 U.S.C. §112, first paragraph rejections of claims 9-12, 14, and 24.

a. Prosecution of present application with respect to rejections under 35 U.S.C. §112, first paragraph fails to meet the Requirements of C.F.R §1.104 and MPEP §2144.03.

b. Prosecution of the present application with respect to rejections under 35 U.S.C. §112, first paragraph fails to meet the Requirements of MPEP §706.07.

6. The Board should withdraw the rejection of claims 9-12, 14, and 24 under 35 U.S.C. §112, first paragraph.

C. Appellant's Claims 9-12, 14, and 24 are Patentable Under 35 U.S.C. § 112, Second Paragraph.

D. Claims 9-12, 14, and 24 are Properly Within the Elected Subject Matter and Should Properly Have Been Considered by the Examiner.

1. The legal requirement for restriction of claims.
2. Election Requirement in the present application.
3. The Board should withdraw the improper restriction of generic claim 9 and require that a reasonable number of species be properly examined.

APPENDIX A CLAIMS INVOLVED IN THE APPEAL

APPENDIX B COPIES OF MATERIALS SUBMITTED TO THE EXAMINER WITH REGARD TO ENABLEMENT

APPENDIX C COPY OF CLAIM 9 AS ORIGINALLY FILED

APPENDIX D COPY OF THE ASSIGNMENT OF THIS INVENTION

APPENDIX E COPY OF EXAMINER'S SEARCH NOTES

The final page of section VII bears the signature of Appellant's attorney.

I. REAL PARTY IN INTEREST

On February 5, 2002 this application was assigned from the inventor, Ben A. Bahr, to the University of Connecticut, a nonprofit organization having a place of business at 263 Farmington Avenue, Farmington, CT 06030. This Assignment was recorded on 02/25/2003 at Reel 013779, frame 0216 for 3 pages. A copy of the Assignment is enclosed as Appendix D.

II. RELATED APPEALS AND INTERFERENCES

There are no known prior or pending appeals, interferences or judicial proceedings related to this application.

III. STATUS OF CLAIMS

a. STATEMENT OF STATUS OF ALL CLAIMS IN THIS PROCEEDING

Claim 13 is allowed.

No claims are objected to.

Claims 9-12, 14, and 24 are rejected.

Claims 1-8 and 15-19 are withdrawn.

Claim 25 has not been entered.

Claims 20-23 are cancelled.

Claims 9-14, 24, and 25 are pending in this application.

b. CLAIMS ON APPEAL

The claims on appeal are claims 9-12, 14, 24 and 25.

IV. STATUS OF AMENDMENTS

On December 28, 2004 Appellant submitted an amendment under Rule 116. The amendment added new claim 25 which was identical to originally filed claim 9 (Appendix C). In the Advisory Action dated February 1, 2005 the Examiner stated that the proposed amendment would not be entered on the basis that new claim 25 raises new issues.

V. SUMMARY OF CLAIMED SUBJECT MATTER

Briefly stated, claim 9 is directed to a method for treating neurodegeneration in a subject suffering from at least one of Alzheimer's disease, Parkinson's disease or lysosomal storage disorders. (page 1, lines 31-32; page 2, lines 3-6; and page 5, line 15 to page 6, line 2). The method includes the administration to a subject of a therapeutically effective amount of a lysosomal modulating compound, a physiologically acceptable salt of the lysosomal modulating compound or a combination thereof, wherein enzymatic capacity of lysosomes in the subject is enhanced. (page 10, lines 18-20; and page 11, line 26 to page 12, line 2).

Briefly stated, claim 25 is directed to a method for treating neurodegeneration in a subject. (page 1, lines 31-32; page 2, lines 3-6; and page 5, line 15 to page 6, line 2). The method includes the administration to the subject a therapeutically effective amount of a lysosomal modulating compound, a physiologically acceptable salt of the lysosomal

modulating compound or a combination thereof, wherein enzymatic capacity of lysosomes in the subject is enhanced (page 10, lines 18-20; and page 11, line 26 to page 12, line 2).

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

A. Whether new claim 25 which was included in Appellant's Rule 116 Amendment filed on December 29, 2004 should be entered.

B. Whether claims 9-12, 14, and 24 are Enabled under 35 U.S.C. §112, first paragraph.

C. Whether claims 9-12, 14, and 24 are Definite under 35 U.S.C. §112, second paragraph.

D. Whether a reasonable number of species should be properly examined.

VIII. ARGUMENT

A. New Claim 25 included in Appellant's Rule 116 Amendment Filed on December 29, 2004 Should be Entered and Considered in this Appeal.

1. The amendment in dispute:

On December 29, 2004 Appellant filed a Response After Final Office Communication (Amendment Under Rule 116). The Response included new claim 25 as follows.

> 25. (new) A method for treating neurodegeneration in a subject, comprising:
>
> administering to the subject a therapeutically effective amount of a lysosomal modulating compound, a physiologically acceptable salt of the lysosomal modulating compound or a combination thereof, wherein enzymatic capacity of lysosomes in the subject is enhanced.

In the Advisory Action mailed on February 1, 2005 the Examiner stated that this amendment would not be entered because "they raise new issues that would require

further consideration and/or search." The Examiner went on state at page 2 of the Advisory Action: "applicant has added claim 25 which raises new issues because it does not qualify the patient as suffering from at least one of Alzheimer's disease, Parkinson's disease or lysosomal storage disorders."

2. Requirements for entry of amended claims under 37 C.F.R. §1.116.

The Manual of Patent Examining Procedure (hereafter MPEP) in section 714.13 recites in part that:

> It should be kept in mind that applicant cannot, as a matter of right...add new claims after final rejection.... Except where an amendment merely cancels claims, adopts examiner suggestions, removes issues for appeal, or in some other way requires only cursory review by the examiner, compliance with the requirement of a showing under 37 C.F.R. 1.116(c) is expected in all amendments after final rejection.... The refusal to enter the proposed amendment should not be arbitrary. The proposed amendment should be given sufficient consideration to determine whether...the issues on appeal are simplified.

3. The record concerning not entered claim 25.

The specification for U.S. Application No. 10/056,666 at page 5, lines 27-29 recites that:

> The novel lysosome modulating compounds can function to enhance cellular production of lysosomal enzymes and thereby promote degradative processing of aberrant protein fragments and aggregates. The digestive processing can be useful in the treatment of neurodegenerative events.

In addition page 11, line 29 to page 12, line 2 recites that:

> The inventive method provides a pronounced increase in lysosomal capacity, to prevent abnormal protein processing, and to attenuate sysnaptic degeneration and dysfunction. The upmodulation of lysosomal enzymes will help offset the accumulation of aberrant proteins found associated with various pathologies and, thus, provide a treatment to delay or slow neurodegenrative events.

Original claim 9 (identical to not entered claim 25) for the 10/056,666 application recites in full:

> A method for treating neurodegeneration in a subject, comprising: administering to the subject a therapeutically effective amount of a lysosomal modulating compound, a physiologically acceptable salt of the lysosomal modulating compound or a combination thereof, wherein enzymatic capacity of lysosomes in the subject is enhanced.

Original claim 9 was rejected in the May 6, 2003 Office action. In addition, original claim 9 was rejected in the October 20, 2003 Office Action made final.

As filed claim 9 was amended to recite, in part, "[a] method for treating neurodegeneration in a subject suffering from at least one of Alzheimer's disease, Parkinson's disease, or lysosomal storage disorders". Amended claim 9 was submitted in the Appellant's January 20, 2004 Response to Office Action, and was not entered based on the Examiner's reasoning, stated in the Office communication of February 27, 2004. That reasoning firmly rested on the assertion that amended claim 9 "raises new issues since the patient has now been qualified as having to suffer from the claimed diseases". However, in apparent contradiction to the "new issues" assertion, the Examiner further asserted, in essence, that the amendment to claim 9 made no patently material change to claim 9 in stating:

> The Seyfried patent does not have to teach the claimed method as now claimed for the above reasons. Thus, the patent does teach a person being administered the claimed composition. The use does not have to be the same as applicant since the amendment is not being entered.

A review of the Examiner's Search Notes in the U.S. Patent and Trademark Office PAIR database for the 10/056,666 application indicates that in March of 2003 the Examiner explicitly searched for prior art references using a search string which included the terms "Lysosom?", "neurodegener?" and "Disease# OR Disorder?" and which search was directed specifically toward original claim 9. Copies of the Examiner's Search Notes are included in Appendix E.

New claim 25, which is identical original claim 9, was included in Appellant's December 29, 2004 Response to Office Action. However, in the Office communication mailed on February 1, 2005, page 2, Continuation of 2, the Examiner refused to enter new claim 25 on the basis that "applicant has added claim 25 which raises new issues because it does not qualify the patient as suffering from at least one of Alzheimer's disease, Parkinson's disease or lysosomal storage disorders (emphasis added)".

4. The Board should enter and consider Appellant's Rule 116 Amendment as filed on December 29, 2004.

Appellant's as filed specification and claims explicitly support treating neurodegeneration with a lysosomal modulating compound wherein enzymatic capacity of lysosomes in the subject is enhanced. The record indicates that the Examiner considered claim 25 in the form of original claim 9. In fact, the Examiner searched for references that encompassed the scope of original claim 9. In addition, the amendment

of original claim 9 to included the limitation that the subject must suffer from "at least one of Alzheimer's disease, Parkinson's disease, or lysosomal storage disorders" was met with the Examiner's assertion that , at best, that amended claim 9 was a subset of original claim 9.

It is clear that when the Examiner was presented with new claim 25, identified on page 11, 2 paragraph 2, 2 line 2 of the December 29, 2004 Response to Office Action as "identical to originally filed claim 9", the Examiner should have immediately understood that entering a claim identical to a claim previously presented and searched claim does not require anything more than a cursory inspection.

It is additionally clear that the Examiner's reasoning in the Office correspondence of February 27, 2004 is contradictory to the "new issues" assertion as contained in the Office communication mailed on February 1, 2005.

It is furthermore clear that Appellant's addition of claim 25, as directly evidenced by the Examiner's search notes indicates that a "new" search was not necessary since the search was previously carried out with regard to identical original claim 9.

As such, for the above reasons, Appellant respectfully requests that the Board enter and consider claim 25 included with the Rule 116 amendment as filed on December 29, 2004.

B. Appellant's Claims 9-12, 14, and 24 are Enabled Under 35 U.S.C. § 112, First Paragraph and are Therefore Patentable.

1. The Legal Burden Necessary to Properly Assert a 35 U.S.C. §112, First Paragraph Rejection

35 U.S.C. §112, first paragraph states: "[t]he specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention. " The courts have clearly stated that in order "[t]o be enabling under §112, a patent must contain a description that enables one skilled in the art to make and use the claimed invention." Amgen Inc. v. Chugai Pharmaceutical Co. Ltd., 18 USPQ2d 1016, 1026 (Fed. Cir. 1991).

The courts have interpreted the enablement requirement to require that the specification teach those in the art to make and use the invention without "undue experimentation". As set out in In re Wands, 858 F.2d 731, 737; 8 USPQ2d 1400, 1404 (Fed. Cir. 1988), factors to be considered in determining whether required experimentation is undue include:

1. The breadth of the claims;
2. The nature of the invention;
3. The state of the prior art;
4. The level of a person of ordinary skill;
5. The level of predictability in the art;
6. The amount of direction provided by the inventor;
7. The existence of working examples in the specification; and
8. The quantity of experimentation needed to make or use the invention based on the content of the disclosure.

The courts have pointed out that "[n]ot every last detail [of an invention need] be described [in a patent specification], else patent specifications would turn into production specifications, which they were never intended to be." In re Gay, 135 USPQ 311,316 (C.C.P.A. 1962). Citing the opinion in Gay, the Board of Patent Appeals and Interferences echoed this point in its statement that " the law does not require a specification to be a blueprint to satisfy the requirement for enablement under 35 U.S.C. 112, first paragraph," Staehelin v. Secher, 24 USPQ2d 1513, 1516 (Bd. Pat. App. & Int. 1992). Even more broadly, the MPEP states the specification need not disclose what is well known to those skilled in the art and preferably omits that which is well known to those skilled and already available to the public. See MPEP section 2164.05(a).

The United States Patent and Trademark Office recognizing the above legal authority has promulgated "Training Materials For Examining Patent Applications With Respect To 35 U.S.C. 112, First Paragraph-Enablement Chemical/Biotechnical Applications". As stated in these training materials at section III, paragraph 6, with bolding added:

> It is improper to conclude that a disclosure is not enabling based on an analysis of only one of the above [Wands] factors while ignoring one or more of the others. The examiner's analysis **must** consider all the evidence related to each of these factors, and any conclusion of non-enablement **must** be based on the evidence as a whole.

In addition, "Enablement is a question of law to be reviewed *de novo*." In re Wright, 27 USPQ2d 1510, 1513 (Fed. Cir 1993). As such, the Board should review the enablement of claims 9-12, 14, and 24.

2. The Asserted Rejections Under 35 U.S.C. §112, First Paragraph.

Claims 9-12, 14, and 24 were rejected under 35 U.S.C. §112, first paragraph, as allegedly not being enabled. The November 5, 2004 Office communication substantially repeats the May 18, 2004 Office communication with regard to the rejection of claims 9-12, 14, and 24.

The Office Communication, mailed November 5, 2004, contains the statement on page 3 that:

> The claims are not enabled for treating Alzheimer's, Parkinson's or lysosomal disorders. There is no known cure for treating Alzheimer's or Parkinson's thus treating is also not known. No known treatment can keep the disorders from happening. In fact, many people in the United States alone have such ailments and never get better. The evidence on record does not show that one who has such ailments ever gets any better, so how can one claim such treatments when they do not do what they claim they do? Without scientific data showing the diseases are prevented or cured then the treatment of diseases is just unknown since patients with such diseases never get any better.
>
> As far as "lysosomal storage disorders" go, they are also not enabled and cannot be determined as to what they would do. Applicant has claimed two specific disorders (Alzheimer's and Parkinson's) but it is not clear what would encompass "lysosomal storage disorders". Without more the claims are simply not enabled by the specification.
>
> Applicant argues that Alzheimer's and Parkinson's have known treatments but are they really effective? The key word is "treating". How can one treat such dieases such as Alzheimer's or Parkinson's if one does not show results *in vivo* that such compounds claimed **effectively** (bolding original) treat the claimed disease[1]. The articles submitted only state that treatments are being tested or speculate that they work, but there is nothing that shows that they **effectively** (bolding original) treat the diseases such as Alzheimer's and Parkinson's.

[1] Appellant, in the Response to Office Action filed on December 29, 2004, respectfully reminded the Examiner that treatment claims do not necessarily require a showing of *in vivo* efficacy to be enabled. See, e.g., Cross v. Iizuka, 753 F.2d 1040, 1051, 224 USPQ 739, 748 (Fed. Cir. 1985).

3. The Office communication assertions do not meet the required legal burden to assert a 35 U.S.C. §112, first paragraph, rejection.

Appellant notes that in properly rejecting claims under 35 U.S.C. §112, first paragraph the Examiner:

> ... bears an initial burden of setting forth a reasonable explanation as to why it believes that the scope of protection provided by that claim is not adequately enabled by the description of the invention provided in the specification of the application; this includes, of course, providing sufficient reasons for doubting any assertions in the specification as to the scope of enablement. In re Wright, 999 F.2d 1557, 1561-62, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993).

In addition, as discussed above the United States Patent and Trademark Office has stated that (bolding added): "It is improper to conclude that a disclosure is not enabling based on an analysis of only one of the above [Wands] factors while ignoring one or more of the others. The examiner's analysis **must** consider all the evidence related to each of these factors . . .

- The Office communications NEVER consider or discuss ANY of the Wands factors at all.
- The Office communications NEVER consider or discuss how the evidence relates to ANY of the Wands factors.

Furthermore, in spite of requests made by the Appellant in the Response to Office Action filed May 18, 2004 and in the Response to Office Action filed August 17, 2004 asking that the Examiner properly include an analysis applying ALL of the Wands factors to present case, the Examiner continued to maintain the rejection of claims 9-12, 14, and

24 under 35 U.S.C. §112, first paragraph **without** the proper analysis or application of the Wands factors.

Therefore, the rejection of claims 9-14 and 24 under 35 U.S.C. § 112, first paragraph should be withdrawn and the claims should be found patentable for at least the reason that the Examiner has failed to properly reject these claims under 35 U.S.C. §112, first paragraph.

a. The Office communication assertions are contrary to knowledge in the art.

The Office communication rejection centers around the position that: "[t]here is no known cure for treating Alzheimer's or Parkinson's thus treating is also not known." November 5, 2004 Office Action, page 3, lines 14-15. More simply, the Office communication appears to assert that cure = treatment. The Office position is therefore misplaced, incorrect, and fundamentally lacking technical support since there are a number of diseases for which no cure is known but for which treatment is possible. For example, it is widely recognized that diabetes cannot be cured, however diabetics can be treated with diet or insulin. As another example, it is widely recognized that HIV cannot be cured, however drugs are available to treat the disease. Thus, it is beyond argument that a disease can be treated even if that same disease cannot be cured. Appellant includes in Appendix B (a-h), as further support for this position, partial copies of United States Patent Nos. 6,605,589; 6,512,003; 6,432,941; 6,251,882; 6,727,265; 6,797,721; 6,743,786; and 6,274,606 which are directed toward treating such disease states as Alzheimer's,

Parkinson's, cancer, and human immunodeficiency virus. Appellant notes that these patents rely on only *in vivo* data.

Clearly, the rejection of claims 9-12, 14, and 24 under 35 U.S.C. § 112, first paragraph, is contrary to the knowledge in the art, and should be found to be improper and the claims found to be patentable for at least this reason and on the prior record.

b. **Appellants Claims Reciting Treatment for Alzheimer's Disease are Enabled.**

The Office position specifically with regard to treatments for, at least, Alzheimer's disease is also misplaced, incorrect and fundamentally lacking technical support. This is evidenced by the plethora of known treatments for Alzheimer's disease. For example, the United States Food and Drug Agency (FDA) specifically stated, as early as 1992, that there are "**[s]everal compounds for the treatment of Alzheimer's disease are under development or testing**. (emphasis added)" FDA's ALZHEIMER'S DISEASE UPDATE, T92-43, page 1, Sept. 24, 1992, (copy found in Appendix B(i)). Furthermore, on September 9, 1993 the Food and Drug Administration approved a drug "**specifically to treat symptoms of Alzheimer's disease** (emphasis added)" and distinguished the term "treatment" from the term "cure" in stating the approved drug was "not a cure for Alzheimer's disease". FDA Press Release, P93-37, September 9, 1993 (copy found in Appendix B(j)). Appellant further notes that as discussed by Dr. Katz in the March 13, 2001 meeting minutes of the Peripheral and Central Nervous System Drugs Advisory Committee (copy found in Appendix B(k)) the "Federal Food, Drug and Cosmetic Act, which is the statute under which we regulate drugs, requires that in order for a new drug

to be approved the sponsor must submit what is called **substantial evidence of effectiveness that the treatment will have the effect represented for it in the product labeling** (emphasis added)...." With regard to Dr. Katz's statement, the Appellant directs attention to the FDA approved labels (copy found in Appendix B(l)) for Exelon® and Aricept® both of which are indicated "for the treatment of mild to moderate dementia of the Alzheimer's type". Unequivocally these labels demonstrate that effective treatments for disease conditions such as Alzheimer's exist. As such, the rejection of claims 9-12, 14, and 24 under 35 U.S.C. §112, first paragraph should be found to be improper and the claims found to be patentable based on the prior record.

c. **The Appellant's Claims Reciting Treatment for Parkinson's Disease are Enabled.**

With specific regard to Parkinson's disease, Appellant directs attention to a published article[2] (copy found in Appendix B(m)) which clearly indicates that as early as, at least, 1998 there existed a "**primary treatment for PD (Parkinson's Disease)** (emphasis added)". Based on at least this showing the Examiner's asserted position with regard to the nonexistence of treatments for Parkinson's disease appears to be in clear error. In addition, and with regard to Dr. Katz's statement above, the Appellant directs attention to the FDA approved label (copy found in Appendix B(n)) for Stalevo® (carbidopa, levodopa and entacapone), indicated "to treat patients with idiopathic Parkinson's disease". Unequivocally this label demonstrates that effective treatments for disease conditions such as Parkinson's exist. Appellant therefore requests that claims 9-14 and 24 be found to

[2] Altered Thalamic Response to Levodopa in Parkinson's Patients With Dopa-induced Dyskinesias, Tamara Hershey et al. Proc. Natl. Acad. Sci. USA, Volume 95, pp 12016-12021, (September 1988).

have been improperly rejected under 35 U.S.C. §112, first paragraph, and the claims found to be patentable for at least these reasons and based on the prior record.

d. Appellants Claims Reciting Treatment for Lysosomal Staorage Disorders are Enabled.

With specific regard to the Examiner's statement in the November 5, 2004 Office Action that "[a]s far as "lysosomal storage disorders" go, they are not enabled and cannot be determined as to what they would do." It has been clearly established that "[p]atent documents are written for persons familiar with the relevant field; the patentee is not required to include in the specification information readily understood by practitioners, lest every patent be required to be written as a comprehensive tutorial and treatise for the generalist, instead of a concise statement for persons in the field. Thus resolution of any ambiguity arising from the claims and specification may be aided by extrinsic evidence of usage and meaning of a term in the context of the invention." Verve, LLC v. Crane Cams, Inc., 311 F.3d 1116, 1119, 65 USPQ2d 1051 (Fed. Cir. 2002); See Bayer AG v. Schein Pharmaceuticals, Inc., 301 F.3d 1306, 1314, 64 USPQ2d 1001 (Fed. Cir. 2002) ("Because an enabling disclosure by definition turns upon the objective understanding of a skilled artisan, the enablement requirement can be met by reference to the knowledge of one of ordinary skill in the relevant art."); S3 Inc. v. nVIDIA Corp., 259 F.3d 1364, 1371, 59 USPQ2d 1745 (Fed. Cir. 2001) ("The law is clear that patent documents need not include subject matter that is known in the field of the invention and is in the prior art, for patents are written for persons experienced in the field of the invention).

The application, as filed, clearly enables the claim since it is well known that lysosomal disruption is associated with, among other things, the intracellular build up of protein fragments and aggregates which in turn are associated with neurodegenerative disorders. Specification, page 1, paragraph 2. In support of this assertion and as discussed by the courts, "the enablement requirement can be met by reference to the knowledge of one of ordinary skill in the relevant art". Bayer AG, at 1314 (Fed. Cir. 2002).

Appellant has therefore included in Appendix B(o-r) several documents and publications[3] which were previously submitted during the prosecution of this application. These documents and publications clearly identify that the phrase "lysosomal storage disorder" was well known in the art at the time the application was filed. Appellant notes that one reference, published in 1998, states that lysosomal storage disorders had been known for at least "a quarter of a century"[4] This reference specifically discusses the effect lysosomal storage disorders have on the intracellular accumulation of metabolic products. One skilled in the relevant art would understand what was encompassed by the phrase "lysosomal storage disorder" and would find the Appellant's specification to provide adequate guidance on how to use the claimed compositions. For example, the Appellant directs attention to at least Example 8 on page 19 of the specification which discloses administering an inventive lysosomal modulator to rats. Appellant notes that Figure 8 of the application illustrates the significant increase of lysosomal enzyme concentration

[3] Appellant directs attention to the following documents found in Appendix B: *Gene Therapy of Lysosomal Storage Disorders*, A. Salvetti, J. M. Heard, O. Danos, British Medical Bulletin, Vol. 51, No. 1, 106-122 (1995); *Prevalence of Lysosomal Storage Disorders*, Peter J. Meikle et al. , Jama, Vol. 281, No.3, 249-254 (1999); *Cellular Pathology of Lysosomal Storage Disorders*, Sidney Weisner, Rose F. Kennedy, Brain Pathology, vol. 8, 175-193 (1998). See also citation and abstracts of 48 items obtained from a search of PubMed for publications prior to the present application's filing date having "lysosomal storage disorders" in the title.

[4] Cellular Pathology of Lysosomal Storage Disorders at page 175.

associated with the administration of the compound. As such, Appellant respectfully requests that the rejection of the claims based on the language "lysosomal storage disorders" be found to be improper and the claims found to be patentable.

e. Appellant's Claims Reciting Lysosomal Storage Disorder are Enabled Based on FDA Approved Labels .

Appellant directs attention to the FDA approved label (copy enclosed) in Appendix B(s) for Aldurazyme® (laronidase) and the toxicologist report indicating that the drug is indicated for a lysosomal storage disease. In light of Dr. Katz's statement, discussed above, this label and report unequivocally demonstrate that effective treatments for conditions such as lysosomal storage disorder exist. As such, the rejections under 35 U.S.C. §112, first paragraph should be found to be improper, and the claims found to be patentable.

f. Appellant's Claims Reciting Lysosomal Storage Disorder are Enabled Based on Recent Scientific Investigation.

In one embodiment Appellant's invention enhances lysosomal function using a lysosomal modulating compound. Appellant previously submitted the cover page from an article published by the Journal of Neuroscience in 2004[5] (copy found in Appendix B(t)). The abstract of this article states that: ". . . enhancing lysosomal function may be a potential therapeutic strategy to halt or even prevent the pathogenesis of Parkinson's disease and other Lewy body diseases." Thus, there is scientific evidence published in medical

[5] Clearance of alpa-Synuclein Oliugomeric Intermediates via the Lysosomal Degradation Pathway, He-Jin Lee, Farnaz Khoshaghideh, Smita Patel and Seung-Jae Lee, The Journal of Neuroscience, 24(8):1888-

journals subsequent to Appellant's filing date supporting the enablement of Appellant's claims. Claims 9-14 and 24 are patentable for at least this reason.

4. The Rejections of Claims 9-12, 14, and 24 are Clearly Arbitrary Based on the Allowance of Claim 13

In the Office communication mailed May 18, 2004, claims 9-14 and 24 were specifically rejected under 35 U.S.C. §112, first paragraph, for at least the reason that "[t]here is no known cure for treating Alzheimer's or Parkinson's thus treating is also not known".

The Office communication of February 1, 2005 contains the statement that claim 13 is "allowed". Claim 13 depends directly from claim 9. However, the communication further asserts that claims 9-12, 14, and 24 are rejected. The sole basis of the 35 U.S.C. §112, first paragraph, rejections was stated in the November 5, 2004 Office communication to be "[t]here is no known cure for treating Alzheimer's or Parkinson's thus treating is also not known". This is the very same basis for which claim 13 had been rejected in the Office communication mail May 18, 2004.

Appellant fails to understand why the previous rejection of claim 13 was withdrawn and claim 13 was allowed while the rejection of 9-12, 14, and 24 were maintained. For example, the rejection of claim 12 has been maintained. Claim 12 depends directly from claim 9. Claim 12 recites, in part, "the method of claim 9, wherein the lysosomal

---

1896 (February 25, 2004).

modulating compound comprises at least one of" six specific compounds or their physiological salts. However, claim 12 has not been allowed. In addition, the rejection of claim 11 has been maintained. Claim 11 depends directly from claim 9. Claim 11 recites, in part, "the method of claim 9, wherein the lysosomal modulating compound comprises" a plurality of structurally defined compounds. However, claim 11 has not been allowed. Furthermore, the rejections of claims 10, 14, and 24 have been maintained. Claims 10, 14, and 24 all depend directly from claim 9. Claim 10, recites, "the method of claim 9, wherein the enhanced enzymatic capacity is sufficient to suppress neuropathogenesis". However, claim 10 has not been allowed. Claim 14, recites, "the method of claim 9, wherein the compound is a selective antagonist for at least one cathepsin enzyme. However, claim 14 has not been allowed. Claim 24, recites, "the method of claim 9, wherein the therapeutically effective amount...is sufficient to enhance enzymatic capacity of lysosomes but is not sufficient to generate pathogenic accumulations of protein or protein fragements". However, claim 24 has not been allowed.

The rejection of independent claim 9 has also been maintained even in light of the allowance of claim 13. Claim 9 recites, in part, "[a] method for treating neurodegeneration in a subject suffering from at least one of Alzheimer's disease, Parkinson's disease or lysosomal storage disorders". Claim 9 has also been specifically rejected under 35 U.S.C. §112, first paragraph for at least the reason that "[t]here is no known cure for treating Alzheimer's or Parkinson's thus treating is also not known".

Based on at least these facts, Appellant cannot find any logical reason for the Examiner's assertions and actions in rejecting claims 9-12, 14, and 24 in view of the Examiner's allowance of claim 13 and the Examiner's stated reasoning.

Therefore, beyond the Examiner's above identified failure to meet the legal burden under 35 U.S.C §112, first paragraph, the Examiner's rejection then subsequent allowance of claim 13 clearly highlights that the Examiner has been arbitrary in rejecting claims 9-12, 14, and 24. As such, the Appellant respectfully asserts that the rejection of claims 9-12, 14, and 24 is improper under 35 U.S.C. §112, first paragraph, and respectfully requests that the claims be found to be patentable.

5. <u>The Office communication of February 1, 2005 is contrary on it face to the development of the issues with respect to the asserted 35 U.S.C. §112, first paragraph, rejection of claims 9-12, 14, and 24.</u>

MPEP §706.07 which relates to Final Rejection discusses the nature of the examination process with regard to development of the issues. For example, §706.07 recites in one particular part:

> [i]n making the final rejection, all outstanding grounds of rejection of record should be carefully reviewed, and any such grounds relied on in the final rejection should be reiterated. They must also be clearly developed to such an extent that Applicant may readily judge the advisability of an appeal unless a single previous Office Action contains a complete statement supporting the rejection...

The Office communication of February 1, 2005 contains the statement that claim 13 is "allowed". However, the communication further asserts that claims 9-12, 14, and 24 are rejected. Appellant notes that the only outstanding rejection of claims 9-12, 14, and 24 rest solely on the basis of non-enablement under 35 U.S.C. §112, first paragraph and on indefiniteness under 35 U.S.C. §112, second paragraph. Appellant additionally notes that the Examiners assertions with regard to non-enablement and indefiniteness are directed toward independent claim 9 on

which allowable claim 13 depends directly. Dependent claim 13 is a method for treating neurodegeneration in a subject suffering from at least on of Alzheimer's disease, Parkinson's disease or lysosomal storage disorders. The assertions of non-enablement and indefiniteness are essentially summed up by the Examiner's statement that "[t]he claims are not enabled for treating Alzheimer's, Parkinson's or lysosomal disorders. There is no known cure for treating Alzheimer's or Parkinson's thus treating is also not known". November 5, 2004 Office communication, page 3.

Allowance of dependent claim 13 clearly is contrary to the development of the issues in the present application since independent claim 9 is rejected for non-enablement and indefiniteness based on the reasoning that "[t]here is no known cure for treating Alzheimer's or Parkinson's thus treating is also not known" November 5, 2004 Office communication, page 3. Appellant therefore requests that the rejection of claims 9-12, 14, and 24 be found to be improper, and the claims found to be patentable based on the prior record.

a. Prosecution of The Present Application with respect to rejections under 35 U.S.C. §112, first paragraph Fails to Meet Requirements of 37 C.F.R. §1.104 and MPEP §2144.03

During prosecution of the present application Appellant submitted copies of statements and materials from the FDA, scientific citations, and other materials (found in Appendix B(u)) showing the Examiner's statement to be in clear error with regard to treatment of Alzheimer's disease, Parkinson's disease, and lysosomal storage disorders. In addition, the Appellant respectfully reminded the Examiner in the December 28, 2004 Response to Office Action that the Administrative Procedure Act (APA) requires that the

Examiner apply a "substantial evidence" standard of review when relying on "common knowledge in the art or well known prior" See MPEP 2144.03. The Appellant further requested that since the Examiner's statements must have been backed by adequate evidence to support a finding that treatment of, at least, Alzheimer's disease is unknown, the Appellant should be furnished with documentary evidence. Since the Examiner failed to provide the Appellant with documentary evidence, the Appellant assumes that the rejection must be based on the Examiner's personal knowledge.

The Appellant requested that the Examiner provide a declaration or affidavit as specified in the MPEP at §2144.03 (C) wherein the Examiner should "provide an affidavit or declaration setting forth specific factual statements and explanation to support" his finding that treatment for Alzheimer's is unknown.

The Examiner failed to address this point and failed to provide the required affidavit or declaration supporting a finding that a treatment of Alzheimer's is unknown. Appellant therefore requests that the rejection of claims 9-14 and 24 be found to be improper. In addition, the Appellant requests that the Board, in finding the rejections of claims 9-14, and 24 to be improper, review the Examiner's rejection with respect to 37 C.F.R. §1.104 Nature of Examination, which reads in one pertinent portion:

> (a) Examiner's action. (1) On taking up an application for examination or a patent in a reexamination proceeding, the Examiner shall make a thorough study thereof and shall make a thorough investigation of the available prior art relating to the subject matter of the claimed invention. The examination shall be complete with respect ...to the patentability of the invention as claimed (emphasis added), as well as with respect to matters of form, unless otherwise indicated.

b. Prosecution of Present Application with respect to rejections under 35 U.S.C. §112, first paragraph Fails to Meet Requirements of MPEP §706.07

Appellant asserts that the Examiner in allowing claim 13, yet rejecting at least claim 9, upon which claim 13 directly depends, under 35 U.S.C. §112, first paragraph, has disregarded MPEP §706.07 which relates to Final Rejection and which reads in one pertinent portion:

> Before final rejection is in order a clear issue should be developed between the Examiner and Applicant. To bring the prosecution to as speedy conclusion as possible and at the same time to deal justly by both the Applicant and the public, the invention as disclosed and claimed should be thoroughly searched in the first Office Action and the references fully applied...
>
> The Applicant who is seeking to define his or her invention in claims that will give him or her the patent protection to which he or she is justly entitled should receive the cooperation of the Examiner to that end, and not be prematurely cut off in the prosecution of his or her case... The Examiner should never lose sight of the fact that in every case the Applicant is entitled to a full and fair hearing, and that a clear issue between Applicant and Examiner should be developed, if possible, before appeal ...
>
> In making the final rejection, all outstanding grounds of rejection of record should be carefully reviewed, and any such grounds relied on in the final rejection should be reiterated. They must also be clearly developed to such an extent that Applicant may readily judge the advisability of an appeal unless a single previous Office Action contains a complete statement supporting the rejection....

Appellant notes that the Examiner has failed to address the reasons for a finding that claims 9-12, 14, and 24 are not enabled under 35 U.S.C. §112, first paragraph when confronted with a showing of substantial evidence by the Appellant. As such, Appellant requests that claims 9-12, 14, and 24 be held to be enabled based on at least the previously submitted materials and found to be patentable based on the prior record.

6. The Board should withdraw the rejection of claims 9-12, 14, and 24 under 35 U.S.C. §112, first paragraph.

In view of the clear disclosure in Appellant's specification, the material submitted to the Examiner in Responses to Office communications, and the Examiner's assertion that claim 13 is allowable, Appellant respectfully asserts that the pending claims would clearly "enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention". As such, Appellant respectfully requests that the Board find the rejection of claims 9-12, 14, and 24 under 35 U.S.C. §112, first paragraph to be improper, and that claims 9-12, 14, and 24 be found to be patentable based on the prior record.

C. Appellant's Claims 9-12, 14, and 24 are Patentable Under 35 U.S.C. § 112, Second Paragraph.

Claims 9-12, 14, and 24 have been rejected under 35 U.S.C. §112, second paragraph, as being indefinite or failing to particularly point and distinctly claim the subject matter which the Appellant regards as the invention. Specifically, the Examiner has stated that [I]t is not clear what is meant by the term, "lysosomal storage disorders". November 5, 2004 Office Action, page 4, second full paragraph.

Appellant notes that "the test for definiteness is whether one skilled in the art would understand the bounds of the claim when read in light of the specification" Miles Laboratory, Inc. v. Shandon Inc., 997 F.2d 870 (Fed. Cir. 1993), cert. denied, 510 U.S. 1100 (1994). It is clear, from the record and the above discussion, what the bounds of the

claim language "lysosomal storage disorders" encompass when read in light of the specification. Clearly one skilled in the relevant art would understand the term "lysosomal storage disorders" and bounds of the claims when read in light of the specification as evidenced by references such as: Gene Therapy of Lysosomal Storage Disorders, A. Salvetti, J. M. Heard, O. Danos, British Medical Bulletin, Vol. 51, No. 1, 106-122 (1995); Prevalence of Lysosomal Storage Disorders, Peter J. Meikle et al. , Jama, Vol. 281, No.3, 249-254 (1999); Cellular Pathology of Lysosomal Storage Disorders, Sidney Weisner, Rose F. Kennedy, Brain Pathology, vol. 8, 175-193 (1998) (copies found in Appendix B). Appellant also directs attention to the citation and abstracts of 48 items obtained from a search of PubMed for publications prior to the application's filing date having "lysosomal storage disorders" in the title (copies found in Appendix B). Since, at least, the term "lysosomal storage disorders" is clearly defined in the art, Appellant respectfully requests that the rejection of claims 9-14 and 24 under 35 U.S.C. §112, second paragraph be found to be improper, and that claims 9-12, 14, and 24 be found to be patentable based on the prior record.

D. Claims 9-12, 14, and 24 are Properly Within the Elected Subject Matter and Should Properly Have Been Considered by the Examiner.

1. <u>The legal requirement for restriction of claims</u>.

As stated in the MPEP §806.04(a), "35 U.S.C. §121 provides that restriction may be required to one of two or more independent and distinct inventions. However, 37 C.F.R. §1.141 provides that a reasonable number of species may still be claimed if the other

conditions of the rule are met". In addition, if an allowable generic claim is present, "more than one species of an invention, not to exceed a reasonable number, may be specifically claimed in different claims in one national application...".

2. Election Requirement in the Present Application.

The Examiner imposed a five way restriction in the present application in the May 6, 2003 Office Action. Group III of the five-way restriction included claim 9-14, "drawn to a method for treating neurodegeneration in a subject, classified in class 423, subclass various". The Office Action further contained the statement that "Applicant is required under 35 U.S.C. 121 to elect a single disclosed species for prosecution on the merits...", and that "[c]urrently, claims 1, 6, 7, 9, 15 and 20 are generic. Appellant elected with traverse group III and the species of claim 13 in the August 1, 2003 Response to Office Action, page 2.

3. <u>The Board should withdraw improper rejection of generic claim 9 and require that a reasonable number of species be properly examined</u>.

Appellant requests that the Board's rejection of generic claim 9 to be improper based on the above discussion. In addition, the Board is requested to require examination of a reasonable number of species as consistent with the MPEP. For example, at least, the 6 species found in claim 12, and/or the species identified in claim 14.

Respectfully submitted,

BEN A. BAHR

By _____
Thomas A. Hatfield
Registration No. 47,637

Date: 4/4/05

Alix, Yale & Ristas, LLP
Attorney for Appellants
750 Main Street
Hartford, CT 06103
(860) 527-9211

Access DB# 89912

SEARCH REQUEST FORM

Scientific and Technical Information Center

Requester's Full Name: Mike Meller Examiner #: 69404 Date: 3/25/03
Art Unit: 1654 Phone Number 30 8-4236 Serial Number: 10/056,666
Mail Box and Bldg/Room Location: 10A03 Results Format Preferred (circle): PAPER DISK E-MAIL

11D13

If more than one search is submitted, please prioritize searches in order of need.

****

Please provide a detailed statement of the search topic, and describe as specifically as possible the subject matter to be searched. Include the elected species or structures, keywords, synonyms, acronyms, and registry numbers, and combine with the concept or utility of the invention. Define any terms that may have a special meaning. Give examples or relevant citations, authors, etc, if known. Please attach a copy of the cover sheet, pertinent claims, and abstract.

Title of Invention: Materials for lysosome Modulation and methods.

Inventors (please provide full names): Ben A. Bahr

Earliest Priority Filing Date: 10/30/2000

**For Sequence Searches Only* Please include all pertinent information (parent, child, divisional, or issued patent numbers) along with the appropriate serial number.*

Please search. claim 9 with respect to using the specific Compound of clm. 13, if no hits, then expand to broader lysosomal comps. as shown in 11.

RECEIVED MAR 25 2003 TECHNICAL INFORMATION CENTER

****

STAFF USE ONLY

Searcher: _____ Point of Contact: Alexandra Waclawiw
Searcher Phone #: _____ Technical Info. Specialist CM1 6A02 Tel: 308-4491
Searcher Location: _____
Date Searcher Picked Up: 3-27
Date Completed: 3-31
Searcher Prep & Review Time: 15
Clerical Prep Time: _____
Online Time: 42

Type of Search
NA Sequence (#) _____
AA Sequence (#) _____
Structure (#) 3
Bibliographic _____
Litigation _____
Fulltext _____
Patent Family _____
Other _____

Vendors and cost where applicable
STN 9 548.06 615
Dialog _____
Questel/Orbit _____ 42
Dr.Link _____
Lexis/Nexis _____
Sequence Systems _____
WWW/Internet _____
Other (specify) _____

PTO-1590 (8-01)

APPENDIX A - CLAIMS INVOLVED IN THE APPEAL

1. (withdrawn) A method of modulating lysosomal function in a subject, comprising:

administering to the subject a therapeutically effective amount of a lysosomal modulating compound, a physiologically acceptable salt of the lysosomal modulating compound, or a combination thereof; wherein the lysosomal modulating compound comprises M-aa_n-CH=N=N, M-aa_n-CH_2-O-CO-[2-R-4-R-6-R-Phenyl] (wherein each R is independently selected), M-aa_n-NH-CH_2-CH=N-NH-CO-NH_2, or M-N=N-CO-CH_2-aa_n-O-R, wherein;

M comprises H, benzyloxycarbonyl ("Z"), succinyl, methyloxysuccinyl, and butyloxycarbonyl;

aa comprises a blocked or unblocked amino acid with the L configuration, D configuration, or no chirality at the alpha-carbon, the amino acid selected from alanine, valine, leucine, isoleucine, proline, methionine, methionine sulfoxide, phenylalanine, tryptophan, glycine, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, phenylglycine, beta-alanine, norleucine, norvaline, alpha-aminobutyric acid, epsilon-aminocaproic acid, citrulline, hydroxyproline, homoarginine, ornithine, sarcosine, indoline 2-carboxylic acid, 2-azetidinecarboxylic acid, pipecolinic acid (2-piperidine carboxylic acid), O-methylserine, O-ethylserine, S-methylcysteine, S-ethylcysteine, S-benzylcysteine, NH_2-CH(CH_2-$CHEt_2$)-COOH, alpha-aminoheptanoic acid, NH_2- CH(CH_2-1-napthyl)-COOH, NH_2-CH(CH_2-2-napthyl)-COOH, NH_2-CH(CH_2-cyclohexyl)-COOH, NH_2-CH(CH_2-cyclopentyl)-COOH, NH_2-CH(CH_2-cyclobutyl)-COOH, NH_2-CH(CH_2-cyclopropyl)-COOH, trifluoroleucine, hexafluoroleucine, phenylalanine with its phenyl mono-, di-, or trisubstituted with K, alanine with its methyl side chain replaced with a lower alkyl side chain, alanine with its methyl side chain replaced with a lower alkyl group with an attached phenyl group, alanine with its methyl side chain replaced with a lower alkyl group with two attached phenyl groups, alanine with its methyl side chain replaced with a lower alkyl group with an attached phenyl group substituted with K, and alanine with its methyl side chain replaced with a lower alkyl group with two attached phenyl groups and at least one phenyl group substituted with K;

n comprises an integer from 1 to about 20;

R comprises H, a lower alkyl group, a lower fluoroalkyl group, benzyl, a lower alkyl group substituted with J, a lower fluoroalkyl group substituted with J, 1-adamantly, 9-fluorenyl, phenyl, phenyl substituted with K, phenyl disubstituted with K, phenyl trisubstituted with K, naphthyl, naphthyl substituted with K, naphthyl disubstituted with K, naphthyl trisubstituted with K, a lower alkyl group with an attached phenyl group, a lower alkyl group with two attached phenyl groups, a lower alkyl group with an attached phenyl group substituted with K, or a lower alkyl group with two attached phenyl groups and at least one phenyl group substituted with K;

J comprises halogen, COOH, OH, CN, NO_2, NH_2, lower alkyl-OH, lower alkoxy, lower alkylamine, di-lower alkylamine, lower alkoxy-CO-, lower alkyl-O-CO-NH, and lower alkyl-S; and

K comprises halogen, lower alkyl, lower alkyl-OH, lower perfluoroalkyl, lower alkoxy, NO_2, CN, OH, CO-OH, amino, lower alkylamine, C2-12 dialkylamine, lower acyl-O-CO-NH, lower alkoxy-CO-, and lower alkyl-S.

2. (withdrawn) The method of claim 1, wherein the lysosomal modulating compound comprises benzyloxycarbonyl-Phe-Ala-diazomethylketone, benzyloxycarbonyl-Phe-Phe-diazomethylketone, benzyloxycarbonyl-Phe-Lys-2,4,6-trimethylbenzoyloxymethylketone, benzyloxycarbonyl-Lys- diazomethylketone, H-Gly-Phe-Gly-aldehyde semicarbazone, diazoacetyl-DL-2-aminohexanoic acid-methyl ester, a physiologically acceptable salt thereof, or a combination thereof.

3. (withdrawn) The method of claim 1, wherein the lysosomal modulating compound comprises benzyloxycarbonyl-Phe-Ala-diazomethylketone.

4. (withdrawn) The method of claim 1, wherein the lysosomal modulating compound is a selective antagonist for at least one cathepsin enzyme.

5. (withdrawn) The method of claim 1, wherein n comprises an integer from 1 to 4.

6. (withdrawn) A method of modulating lysosomal function in a subject, comprising:

administering to the subject a therapeutically effective amount of a lysosomal modulating compound selected from peptidyl diazomethylketones, peptidyl semicarbazones, diazoacetyl peptidyl alkyl esters, and physiologically acceptable salts thereof.

7. (withdrawn) A method of reducing the risk of neurodegeneration in a subject, comprising:

administering to the subject a therapeutically effective amount of a lysosomal modulating compound, a physiologically acceptable salt of the lysosomal modulating compound, or a combination thereof, wherein enzymatic capacity of lysosomes in the subject is enhanced.

8. (withdrawn) The method of claim 7, wherein the lysosomal modulating compound comprises M-aa_n-CH=N=N; M-aa_n-CH_2-O-CO-[2-R-4-R-6-R-Phenyl] (wherein each R is independently selected); M-aa_n-NH-CH_2-CH=N-NH-CO-NH_2; or M-N=N-CO-CH_2-aa_n-O-R, wherein;

M comprises H, benzyloxycarbonyl ("Z"), succinyl, methyloxysuccinyl, and butyloxycarbonyl;

aa comprises a blocked or unblocked amino acid with the L configuration, D configuration, or no chirality at the alpha-carbon, the amino acid selected from alanine, valine, leucine, isoleucine, proline, methionine, methionine sulfoxide, phenylalanine, tryptophan, glycine, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, phenylglycine, beta-alanine, norleucine, norvaline, alpha-aminobutyric acid, epsilon-aminocaproic acid, citrulline, hydroxyproline, homoarginine, ornithine, sarcosine, indoline 2-carboxylic acid, 2-azetidinecarboxylic acid, pipecolinic acid (2-piperidine carboxylic acid), O-methylserine, O-ethylserine, S-methylcysteine, S-ethylcysteine, S-benzylcysteine, NH_2-CH(CH_2-$CHEt_2$)-COOH, alpha-aminoheptanoic acid, NH_2- CH(CH_2-1-napthyl)-COOH, NH_2-CH(CH_2-2-napthyl)-COOH, NH_2-CH(CH_2-cyclohexyl)-COOH, NH_2-CH(CH_2-cyclopentyl)-COOH, NH_2-CH(CH_2-cyclobutyl)-COOH, NH_2-CH(CH_2-cyclopropyl)-COOH, trifluoroleucine, hexafluoroleucine,

phenylalanine with its phenyl mono-, di-, or trisubstituted with K, alanine with its methyl side chain replaced with a lower alkyl side chain, alanine with its methyl side chain replaced with a lower alkyl group with an attached phenyl group, alanine with its methyl side chain replaced with a lower alkyl group with two attached phenyl groups, alanine with its methyl side chain replaced with a lower alkyl group with an attached phenyl group substituted with K, and alanine with its methyl side chain replaced with a lower alkyl group with two attached phenyl groups and at least one phenyl group substituted with K;

n comprises an integer from 1 to about 20;

R comprises H, a lower alkyl group, a lower fluoroalkyl group, benzyl, a lower alkyl group substituted with J, a lower fluoroalkyl group substituted with J, 1-adamantyl, 9-fluorenyl, phenyl, phenyl substituted with K, phenyl disubstituted with K, phenyl trisubstituted with K, naphthyl, naphthyl substituted with K, naphthyl disubstituted with K, naphthyl trisubstituted with K, a lower alkyl group with an attached phenyl group, a lower alkyl group with two attached phenyl groups, a lower alkyl group with an attached phenyl group substituted with K, or a lower alkyl group with two attached phenyl groups and at least one phenyl group substituted with K;

J comprises halogen, COOH, OH, CN, NO_2, NH_2, lower alkyl-OH, lower alkoxy, lower alkylamine, di-lower alkylamine, lower alkoxy-CO-, lower alkyl-O-CO-NH, and lower alkyl-S-; and

K comprises halogen, lower alkyl, lower alkyl-OH, lower perfluoroalkyl, lower alkoxy, NO_2, CN, OH, CO-OH, amino, lower alkylamine, C2-12 dialkylamine, lower acyl-O-CO-NH, lower alkoxy-CO-, and lower alkyl-S.

9. (previously presented) A method for treating neurodegeneration in a subject suffering from at least one of Alzheimer's disease, Parkinson's disease or lysosomal storage disorders, comprising:

administering to the subject a therapeutically effective amount of a lysosomal modulating compound, a physiologically acceptable salt of the lysosomal modulating compound or a combination thereof, wherein enzymatic capacity of lysosomes in the subject is enhanced.

10. (original) The method of claim 9, wherein the enhanced enzymatic capacity is sufficient to suppress neuropathogenesis.

11. (original) The method of claim 9, wherein the lysosomal modulating compound comprises M-aa_n-CH=N=N; M-aa_n-CH_2-O-CO-[2-R-4-R-6-R-Phenyl] (wherein each R is independently selected); M-aa_n-NH-CH_2-CH=N-NH-CO-NH_2; M-N=N-CO-CH_2-aa_n-O-R; wherein;

M comprises H, benzyloxycarbonyl ("Z"), succinyl, methyloxysuccinyl, and butyloxycarbonyl;

aa comprises a blocked or unblocked amino acid with the L configuration, D configuration, or no chirality at the alpha-carbon, the amino acid selected from alanine, valine, leucine, isoleucine, proline, methionine, methionine sulfoxide, phenylalanine, tryptophan, glycine, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, phenylglycine, beta-alanine, norleucine, norvaline, alpha-aminobutyric acid, epsilon-aminocaproic acid, citrulline, hydroxyproline, homoarginine, ornithine, sarcosine, indoline 2-carboxylic acid, 2-azetidinecarboxylic acid, pipecolinic acid (2-piperidine carboxylic acid), O-methylserine, O-ethylserine, S-methylcysteine, S-ethylcysteine, S-benzylcysteine, NH_2-CH(CH_2-$CHEt_2$)-COOH, alpha-aminoheptanoic acid, NH_2- CH(CH_2-1-napthyl)-COOH, NH_2-CH(CH_2-2-napthyl)-COOH, NH_2-CH(CH_2-cyclohexyl)-COOH, NH_2-CH(CH_2-cyclopentyl)-COOH, NH_2-CH(CH_2-cyclobutyl)-COOH, NH_2-CH(CH_2-cyclopropyl)-COOH, trifluoroleucine, hexafluoroleucine, phenylalanine with its phenyl mono-, di-, or trisubstituted with K, alanine with its methyl side

chain replaced with a lower alkyl side chain, alanine with its methyl side chain replaced with a lower alkyl group with an attached phenyl group, alanine with its methyl side chain replaced with a lower alkyl group with two attached phenyl groups, alanine with its methyl side chain replaced with a lower alkyl group with an attached phenyl group substituted with K, and alanine with its methyl side chain replaced with a lower alkyl group with two attached phenyl groups and at least one phenyl group substituted with K;

n comprises an integer from 1 to about 20;

R comprises H, a lower alkyl group, a lower fluoroalkyl group, benzyl, a lower alkyl group substituted with J, a lower fluoroalkyl group substituted with J, 1-adamantyl, 9-fluorenyl, phenyl, phenyl substituted with K, phenyl disubstituted with K, phenyl trisubstituted with K, naphthyl, naphthyl substituted with K, naphthyl disubstituted with K, naphthyl trisubstituted with K, a lower alkyl group with an attached phenyl group, a lower alkyl group with two attached phenyl groups, a lower alkyl group with an attached phenyl group substituted with K, or a lower alkyl group with two attached phenyl groups and at least one phenyl group substituted with K;

J comprises halogen, COOH, OH, CN, NO_2, NH_2, lower alkyl-OH, lower alkoxy, lower alkylamine, di-lower alkylamine, lower alkoxy-CO-, lower alkyl-O-CO-NH, and lower alkyl-S; and

K comprises halogen, lower alkyl, lower alkyl-OH, lower perfluoroalkyl, lower alkoxy, NO_2, CN, OH, CO-OH, amino, lower alkylamine, C2-12 dialkylamine, lower acyl-O-CO-NH, lower alkoxy-CO-, and lower alkyl-S.

12. (original) The method of claim 9, wherein the lysosome modulating compound comprises at least one of benzyloxycarbonyl-Phe-Ala-diazomethylketone, benzyloxycarbonyl-Phe-Phe-diazomethylketone, benzyloxycarbonyl-Phe-Lys-2,4,6-trimethylbenzoyloxymethylketone, benzyloxycarbonyl-Lys- diazomethylketone, H-Gly-Phe-Gly-aldehyde semicarbazone, diazoacetyl-DL-2-aminohexanoic acid-methyl ester or physiologically acceptable salts thereof.

13. (original) The method of claim 9, wherein the compound comprises benzyloxycarbonyl-Phe-Ala-diazomethylketone or physiologically acceptable salts thereof.

14. (original) The method of claim 9, wherein the compound is a selective antagonist for at least one cathepsin enzyme.

15. (withdrawn) A pharmaceutical preparation, including:

at least one lysosomal modulating compound, a physiologically acceptable salt of the lysosomal modulating compound, or a combination thereof; wherein the lysosomal modulating compound comprises M-aa_n-CH=N=N; M-aa_n-CH_2-O-CO-[2-R-4-R-6-R-Phenyl] (wherein each R is independently selected); M-aa_n-NH-CH_2-CH=N-NH-CO-NH_2; M-N=N-CO-CH_2-aa_n-O-R, wherein;

M comprises H, benzyloxycarbonyl ("Z"), succinyl, methyloxysuccinyl, and butyloxycarbonyl;

aa comprises a blocked or unblocked amino acid with the L configuration, D configuration, or no chirality at the alpha-carbon, the amino acid selected from alanine, valine, leucine, isoleucine, proline, methionine, methionine sulfoxide, phenylalanine, tryptophan, glycine, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, phenylglycine, beta-alanine, norleucine, norvaline, alpha-aminobutyric acid, epsilon-aminocaproic acid, citrulline, hydroxyproline, homoarginine, ornithine, sarcosine, indoline 2-carboxylic acid, 2-azetidinecarboxylic acid, pipecolinic acid (2-piperidine carboxylic acid), O-methylserine, O-ethylserine, S-methylcysteine, S-ethylcysteine, S-benzylcysteine, NH_2-CH(CH_2-$CHEt_2$)-COOH, alpha-aminoheptanoic acid, NH_2- CH(CH_2-1-napthyl)-COOH, NH_2-CH(CH_2-2-napthyl)-COOH, NH_2-CH(CH_2-cyclohexyl)-COOH, NH_2-CH(CH_2-cyclopentyl)-COOH, NH_2-CH(CH_2-cyclobutyl)-COOH, NH_2-CH(CH_2-cyclopropyl)-COOH, trifluoroleucine, hexafluoroleucine, phenylalanine with its phenyl mono-, di-, or trisubstituted with K, alanine with its methyl side chain replaced with a lower alkyl side chain, alanine with its methyl side chain replaced with a lower alkyl group with an attached phenyl group, alanine with its methyl side chain replaced with a lower alkyl group with two attached phenyl groups, alanine with its methyl

side chain replaced with a lower alkyl group with an attached phenyl group substituted with K, and alanine with its methyl side chain replaced with a lower alkyl group with two attached phenyl groups and at least one phenyl group substituted with K;

n comprises an integer from 1 to about 20;

R comprises H, a lower alkyl group, a lower fluoroalkyl group, benzyl, a lower alkyl group substituted with J, a lower fluoroalkyl group substituted with J, 1-adamantyl, 9-fluorenyl, phenyl, phenyl substituted with K, phenyl disubstituted with K, phenyl trisubstituted with K, naphthyl, naphthyl substituted with K, naphthyl disubstituted with K, naphthyl trisubstituted with K, a lower alkyl group with an attached phenyl group, a lower alkyl group with two attached phenyl groups, a lower alkyl group with an attached phenyl group substituted with K, or a lower alkyl group with two attached phenyl groups and at least one phenyl group substituted with K;

J comprises halogen, COOH, OH, CN, NO_2, NH_2, lower alkyl-OH, lower alkoxy, lower alkylamine, di-lower alkylamine, lower alkoxy-CO-, lower alkyl-O-CO-NH, and lower alkyl-S-; and

K comprises halogen, lower alkyl, lower alkyl-OH, lower perfluoroalkyl, lower alkoxy, NO_2, CN, OH, CO-OH, amino, lower alkylamine, C2-12 dialkylamine, lower acyl-O-CO-NH, lower alkoxy-CO-, and lower alkyl-S-.

16. (withdrawn) The pharmaceutical preparation of claim 15, wherein the compound is administered to a subject at a therapeutically effective amount to modulate cellular content of lysosomes.

17. (withdrawn) The pharmaceutical preparation of claim 15, wherein the compound comprises at least one of benzyloxycarbonyl-Phe-Ala-diazomethylketone, benzyloxycarbonyl-Phe-Phe-diazomethylketone, benzyloxycarbonyl-Phe-Lys-2,4,6-trimethylbenzoyloxymethylketone, benzyloxycarbonyl-Lys- diazomethylketone, H-Gly-Phe-Gly-aldehyde semicarbazone, diazoacetyl-DL-2-aminohexanoic acid-methyl ester, or physiologically acceptable salts thereof.

18. (withdrawn) The pharmaceutical preparation of claim 15, wherein the compound comprises benzyloxycarbonyl-Phe-Ala-diazomethylketone or physiologically acceptable salts thereof.

19. (withdrawn) The pharmaceutical preparation of claim 15, wherein the compound is a selective antagonist for at least one cathepsin enzyme.

20-23 (cancelled)

24. (previously presented) The method of claim 9 wherein the therapeutically effective amount of a lysosomal modulating compound, a physiologically acceptable salt of the lysosomal modulating compound or a combination thereof administered to the subject is sufficient to enhance enzymatic capacity of lysosomes but is not sufficient to generate pathogenic accumulations of proteins or protein fragments.

25. (Not Entered) A method for treating neurodegeneration in a subject, comprising: administering to the subject a therapeutically effective amount of a lysosomal modulating compound, a physiologically acceptable salt of the lysosomal modulating compound or a combination thereof, wherein enzymatic capacity of lysosomes in the subject is enhanced.

Answers 09/24/1992

T92-43
Sept. 24, 1992

Faye Peterson
(301) 443-3285

ALZHEIMER'S DISEASE UPDATE

FDA has received inquiries about the status of therapies for Alzheimer's disease. (See talk papers T87-52, T88-27, T91-39 and T91-75.) Alzheimer's is a degenerative and fatal disease that follows a progressive course over a period of several years. It affects an estimated 4 million Americans.

The agency has approved a treatment IND for one Alzheimer's therapy -- tacrine or THA (Cognex) -- and is considering a New Drug Application for another. Health and Human Services funding for research related to Alzheimer's disease has more than doubled -- from $130.7 million in 1989 to a projected $293.5 million in 1993. In addition, officials have met with patient advocacy groups and sponsored workshops and other scientific forums to encourage further research.

The following may be used to answer questions:

Several compounds for the treatment of Alzheimer's disease are under development or testing. Among them are a group of compounds that mimic or enhance the effects of acetylcholine, affecting brain nerve pathways known to degenerate to a greater degree in Alzheimer's patients. Tacrine is one of these compounds.

In 1986 tacrine received attention as a treatment for Alzheimer's disease, based on a study reported in the New England Journal of Medicine. While the report was encouraging, the study involved only a very small

-MORE-

number of patients, and much remained unknown about the drug's efficacy and safety, especially its potential for severe liver toxicity.

In July 1991, FDA's independent advisory committee met to consider an application from the Warner-Lambert Co. for approval of tacrine for patients with Alzheimer's disease. The committee recommended that the drug not be approved at that time on the basis of available data but that further studies be conducted and expanded access afforded through a Treatment IND.

In December 1991, FDA approved the Treatment IND on the basis that the drug at low doses appeared to produce a small improvement in mental function in some patients and that there was some reason to believe larger doses, which were under study, might be more effective. The Treatment IND is subject to monitoring and recordkeeping and requires that further controlled studies also be conducted.

The Treatment IND may enroll up to 15,000 patients who receive the drug at increasingly higher doses, up to a maximum of 120 mg. per day. Approximately 2,800 patients are currently enrolled. Since data on the efficacy of the drug at doses of 40 and 80 mg. were not convincing, the accompanying controlled trial will include doses of up to 160 mg., in an attempt to determine if they would be helpful in treating some or all patients. Results of the controlled trial are expected to be available within the next year.

One other New Drug Application for an Alzheimer's drug -- velnacrine (Mentane), developed and tested by Hoechst-Roussel Co., of Somerville, N.J. -- has been submitted to FDA.

The agency is keenly aware of certain patients' desires to import and use unapproved drugs for treating Alzheimer's and has therefore allowed such "personal use" importations of tacrine on a case-by-case basis. These

-MORE-

have been allowed on a one-time basis with assurances by the patient and physician that the patient will be closely monitored to detect potential serious side effects. However, the agency discourages the importation of tacrine from unapproved sources, preferring instead that patients be encouraged to enroll in the treatment IND.

FDA Commissioner David A. Kessler, M.D., met with representatives of Alzheimer's patient advocate organizations on Sept. 9, 1992. Dr. Kessler reiterated his commitment to providing expanded access to tacrine, as long as patients are monitored appropriately and programs are conducted so that data can be collected to evaluate the drug's safety and effectiveness.

FDA is looking forward to increased contact with these and other Alzheimer's groups. Based on its experience with AIDS and cancer patient advocacy groups, the agency believes that more communication will lead to increased mutual understanding and progress in dealing with this life-threatening disease.

NEWS 09/09/1993

P93-37
FOR IMMEDIATE RELEASE

Food and Drug Administration
Susan Cruzan (301) 443-3285

The Food and Drug Administration today announced the approval of tacrine hydrochloride, the first drug approved specifically to treat symptoms of Alzheimer's disease.

Alzheimer's disease, a progressive condition affecting memory, judgment and the ability to reason, affects an estimated four million Americans. Tacrine was found in two controlled trials to provide a small but clinically meaningful benefit for some patients with mild to moderate Alzheimer's disease.

"Tacrine is the first drug shown to have some effect on the disease's devastating symptoms," said FDA Commissioner David A. Kessler, M.D. "It is not a cure for Alzheimer's disease, but it provides some relief for patients and their families."

The conclusion that tacrine is effective for the treatment of symptoms of mild to moderate Alzheimer's disease was based on studies showing that the drug is superior to a placebo in influencing measurements designed to assess antidementia drugs. These measurements include a specific performance test that assesses memory and reasoning ability, and an overall assessment of function based on an interview by a trained clinician.

-MORE-

An advisory panel recommended approval of tacrine in March 1993 based on new studies submitted by the manufacturer, Warner-Lambert Co. of Morris Plains, N.J., which will market the drug under the trade name Cognex. The firm conducted these studies after an advisory committee concluded in July 1991 that the available evidence based on relatively low doses did not support approval of the drug. The committee recommended that the company study use of higher doses over a longer period of time. In one of the new studies, which lasted 30 weeks, patients were given between 80 and 160 mg. of the drug.

Today's approval comes seven months after the manufacturer submitted data using the higher doses.

Because tacrine can cause mild liver toxicity, which has been reversible if tacrine treatment is withdrawn promptly, the labeling for the drug recommends an escalating dosing regimen with frequent blood tests in order to identify patients sensitive to the drug. In patients experiencing mild liver toxicity, it is often possible to continue at a lower dose or stop and then resume therapy at a lower dose. Other side effects include nausea, vomiting, diarrhea and rash.

Tacrine has been available since February 1992 to patients under a "treatment IND" protocol that has permitted more than 7,400 patients to receive the drug while the controlled clinical studies were being completed. IND stands for "investigational new drug."

FDA is one of eight Public Health Service agencies within HHS.

####

DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

CENTER FOR DRUG EVALUATION AND RESEARCH

PERIPHERAL AND CENTRAL NERVOUS SYSTEM DRUGS ADVISORY COMMITTEE

VOLUME I

Tuesday, March 13, 2001

8:00 a.m.

Holiday Inn Gaithersburg
Two Montgomery Village Avenue
Gaithersburg, Maryland

PARTICIPANTS

Claudia H. Kawas, M.D., Consultant and Acting Chairperson
Sandra Titus, Executive Secretary

MEMBERS:

LaRoy P. Penix, M.D.
Gerald Van Belle, Ph.D.
Howard L. Weiner, M.D.
Michael Grundman, M.D., M.P.H.
Jerry S. Wolinsky, M.D.

INVITED SPEAKERS:

Helena Chui, M.D.
Steven DeKosky, M.D.
Ranjan Duara, M.D.
Steven Ferris, M.D.
Mary Ganguli, M.D.
Ronald Petersen, M.D., Ph.D.

PUBLIC SPEAKERS:

Dr. Barry Reisberg
Dr. Tony Waegeman
Dr. Yogesh Shah

FDA:

Robert Temple, M.D.
Russell Katz, M.D.
Ranjit Mani, M.D.

C O N T E N T S

Call to Order and Introduction, Claudia Kawas, M.D. 4

Conflict of Interest Statement, Sandra Titus, Ph.D. 5

Welcome and FDA Overview of Issues, Russell Katz, M.D. 9

Presentations and Discussion on Mild Cognitive Impairment (MCI) and Drug Development Issues:

Mild Cognitive Impairment: Unresolved Issues, Ronald Petersen, M.D. 16

Comments, Steven DeKosky, M.D. 48

Factors that Modify Conversion Rates of MCI in a Clinic-Based and a Community-Based Study, Ranjan Duara, M.D. 69

Mild Cognitive Impairment as a Target for Drug Development, Steven Ferris, Ph.D. 81

Mild Cognitive Impairment: A View from the Trenches, Mary Ganguli, M.D. 117

Clinical Trial Designs for MCI, Michael Grundman, M.D., M.P.H. 139

Public Speakers:

Mild Cognitive Impairment: a Broad Perspective, Barry Reisberg, M.D., IPA 160

MCI is a Clinical Entity: Overview of Design Issues, Tony Waegeman, M.D., UCB Pharma 182

Speed of Processing, the Missing Measure in the Detection of MCI, Yogesh Shah, M.D. 192

Committee Discussion and Deliberation 200

PROCEEDINGS

Call to Order and Introductions

DR. KAWAS: Good morning, and welcome to our meeting of the Peripheral and Central Nervous System Drug Advisory Committee. My name is Claudia Kawas. I am from the University of California at Irvine, and we will now call the meeting to order.

If we can begin first with introductions so everyone will know who is seated around the table, perhaps we can start with the FDA in the corner. Dr. Katz?

DR. KATZ: Russ Katz, Division of Neuropharmacological Drug Products at the agency.

DR. MANI: Ranjit Mani, Division of Neuropharm.

DR. PENIX: LaRoy Penix, Moorehouse School of Medicine, Neuroscience Institute.

DR. VAN BELLE: Gerald Van Belle, University of Washington in Seattle.

DR. WEINER: Howard Weiner, Brigham and Women's Hospital, Harvard Medical School.

DR. WOLINSKY: Jerry Wolinsky, University of Texas, Houston.

DR. GRUNDMAN: Michael Grundman, University of California, San Diego.

DR. TITUS: Sandy Titus, the FDA. I am the executive secretary for this committee.

DR. PETERSEN: Ron Petersen, Mayo Clinic, Rochester, Minnesota.

DR. GANGULI: Mary Ganguli, University of Pittsburgh.

DR. DUARA: Ranjan Duara, University of Miami School of Medicine.

DR. DEKOSKY: Steven DeKosky, University of Pittsburgh.

DR. FERRIS: Steven Ferris, New York University School of Medicine.

DR. KAWAS: Thank you very much. I think we have a very interesting day. We will now let Dr. Titus read the conflict of interest statement.

Conflict of Interest Statement

DR. TITUS: The following announcement addresses the issue of conflict of interest with regard to this meeting and is made a part of the record to preclude even the appearance of such at this meeting.

Based on the submitted agenda for the meeting and all financial interests reported by the committee participants, it has been determined that all interests in firms regulated by the Center for Drug Evaluation and Research which have been reported by the participants present no potential for an appearance of a conflict of interest at this meeting with the following exceptions:

Since the issue to be discussed by the committee at this meeting will not have a unique impact on any particular firm or product but, rather, may have widespread implications with respect to an entire class of products, in accordance with USC 208(b), each participant has been granted a waiver which permits them to participate in today's discussions.

A copy of these waiver statements may be obtained by submitting a written request to agency's Freedom of Information Office, Room 12A-30 of the Parklawn Building.

With respect to FDA's invited guests, there are reported interests which we believe should be made public to allow the participants to objectively evaluate their comments. Dr. Ronald Petersen would like to disclose that he is project director on a National Institute of Aging grant which is supported by Pfizer, Eisai and Roche Vitamins.

Dr. Philip Gorelick would like to disclose that he has two NIH grants. Roche Laboratories and Bayer supplies the medication for each of these grants. In addition, he is on the speaker bureaus for Janssen/Excerpta Medica, Dupont, Roche Laboratories, Bristol Myers Squibb and Boehringer Ingelheim. Dr. Gorelick has consultant agreements with NPS, Eisai, G.D. Searle/Lorex, Roche Laboratories, Ketchum, AstraZeneca, Glaxo Wellcome, Warner-Lambert, Baxter, Rand, Solvay Pharmaceutical and Consumer Healthcare Products

Association. He is also on the Through Leader Panel which is supported by the Weinberg Group.

Dr. Ranjan Duara would like to disclose that he is an investigator on a study entitled Validations of a Memory Screening Instrument. The study is supported by a contract from Pfizer. He also serves as a scientific advisor for Pfizer/Eisai, Novartis and Janssen.

Dr. Steven DeKosky would like to report that he owns stock in Cephalon. He is a research investigator for Eisai-Pfizer, Novartis, and Schwabe. In addition, Dr. DeKosky consults for Pfizer, Cephalon, Schwabe, Janssen, Novartis, AstraZeneca and Eli Lilly, and serves as a speaker for Novartis.

Finally, Dr. Mary Ganguli would like to report that she is a researcher for the National Institutes of Health.

In the event that the discussions involve any other products or firms not already on the agenda for which an FDA participant has a financial interest, the participants are aware of the need to exclude themselves from such involvement and their exclusions will be noted for the record.

With respect to all other participants, we ask in the interest of fairness that they address any current or previous financial involvement with any firm whose products

they may wish to comment upon. Thank you.

DR. KAWAS: Thank you, Dr. Titus. I think Dr. Temple just joined us. Maybe we can let him introduce himself.

DR. TEMPLE: I am Dr. Temple. I am director of this Office in which Neuropharm is.

DR. KAWAS: This committee was convened in order to discuss the topic of MCI or mild cognitive impairment. We have an awful lot of material that is going to be presented today by an awful lot of people. I am told I am supposed to be up here with a timer that has fifteen minutes for each of you to speak and five minutes of questions, and that is going to be the challenge of the day. There is a light up there for the speakers. You will have a two-minute warning when the light will become yellow. After that Sandy gets up on the table and starts making signs if you go beyond.

I wanted us to have a lot of time for discussion. So, we are going to try and keep the presentations as much on schedule as possible, realizing that some of the discussion might happen in the middle of presentations. By unanimous opinion and coercion, Dr. Ron Petersen has been moved into the first speaker slot. So, without further ado, Dr. Petersen, Mayo Clinic, Department of Neurology. Oh, we left out Dr. Katz.

[Laughter]

We really do want to give FDA their time to tell us our mission for today. So, Dr. Russell Katz?

Welcome and FDA Overview of Issues

DR. KATZ: First of all, good morning. I would like to welcome the committee to this meeting, the PCNS advisory committee. I would particularly like to extend an additional welcome to our invited guests who have agreed to graciously give their time and their expertise to help us out this morning. Let me also thank Sandy Titus for arranging the meeting, and I would particularly let me thank explicitly Dr. Ranjit Mani, a medical reviewer in the Division, who is sitting at the table, who really pretty much put the meeting together, identified the experts who are here today, invited them, and pretty much wrote the briefing memo in the books that you have received for today's and tomorrow's meeting. So, thanks, Ranjit.

We are actually presenting you with a fairly unusual problem today. Ordinarily we would bring to the committee a particular application for a new drug and we would ask you to interpret the data and help us out there, but today we are asking you a very different sort of question, a more difficult question, it seems to me. We are asking you to address some fundamental aspects of a particular diagnosis to help us characterize, decide if it

exists and how best it ought to be studied. That is unusual and we know it is difficult.

The reason we are asking now is because a number of pharmaceutical sponsors have approached the Division, asking to develop treatments for mild cognitive impairment or MCI. MCI, as you know, has been characterized variously in the literature but, in general, it is a condition that is described as occurring in elderly patients who predominantly have a memory impairment, some slight cognitive impairment perhaps and some minimal dysfunction in their daily functioning, although that is generally relatively intact, and patients are considered neither to be normal nor to have dementia but their cognitive status falls somewhere in between.

Most of the trials that the sponsors have come to us with have identified as a primary measure of drug effect time to progression to Alzheimer's disease, although some of them look strictly at the symptoms of MCI. We have let these trials proceed but we have told all sponsors that we will not make any commitments as far as interpreting the data pending a wider discussion of some of these more fundamental questions that I hope we will work out or at least discuss today.

By way of background, let me just say that the Federal Food, Drug and Cosmetic Act, which is the statute

under which we regulate drugs, requires that in order for a new drug to be approved the sponsor must submit what is called substantial evidence of effectiveness that the treatment will have the effect represented for it in product labeling. It is important to understand that a product's approval is inextricably linked to the language that is used in product labeling. I say this because one of the most critical factors that we need to consider when we are considering approving a drug and, therefore, writing labeling for it is whether or not the population for whom the drug is intended can be unambiguously described.

So, that takes us to the first question we would like you to think about. In the case of MCI there is not unanimity in the literature about the diagnostic criteria that can reliably identify patients who are alleged to have the condition. So, as I say, one of the critical questions we would like you to address is whether or not you believe that there do exist a set of criteria that can be readily applied by practitioners and that can reproducibly and reliably identify patients presumed to have MCI.

Ordinarily, diagnostic criteria are ideally compared to a gold standard to decide how specific and sensitive they are. Obviously, for example, in Alzheimer's disease the clinical criteria can be validated against the pathologic findings and they do pretty well, as you know,

against those. But, given the nature of MCI, there isn't this wide, robust pathologic database against which to compare the diagnostic criteria. So, that is a particular complication here.

Even if you find that there is a specific set of diagnostic criteria that can reliably identify patients as having MCI, there is another very critical question we would like you to address, and I guess it will take up a good part of the discussion this afternoon. In longitudinal studies of patients diagnosed with MCI, a substantial proportion of those patients go on to progress to frank Alzheimer's disease, and I expect that later today we will hear various estimates about the probability of that happening in these cohorts. In addition, static and functional imaging studies in patients diagnosed with MCI reveal changes that are basically qualitatively similar to those seen in Alzheimer's patients, though quantitatively much less severe, and the few pathologic studies that have been done in these patients also reveal qualitatively similar changes as those seen in patients with Alzheimer's disease.

These factors, taken together, suggest that MCI may, in fact, just simply be early Alzheimer's disease in patients who have not yet progressed to the point where they meet the formal, accepted clinical criteria for making that diagnosis. So, we are particularly interested in your views

on whether or not you think MCI really is just early Alzheimer's disease. It is critical because if it is early Alzheimer's disease it would be inappropriate to grant a claim for the indication of MCI when, in fact, it really is something else.

As you probably know, currently there are four approved treatments for Alzheimer's disease and for what we call mild to moderate Alzheimer's disease, and it is fair to ask if a drug is shown to be effective in patients diagnosed we MCI, if that is fundamentally different from the claims that we have already granted to these four drugs.

In fact, as I said earlier, the trial design that we have most commonly seen for these patients looks, as a primary measure of drug effectiveness, at time to diagnosis of Alzheimer's disease. So, that design itself could be taken to suggest that, in fact, these patients really just have an early stage of that condition.

It is also true that in the longitudinal studies which document progression to Alzheimer's disease in some proportion of patients that there is some proportion of patients who don't progress to Alzheimer's disease. That might possibly be an artifact of the fact that the follow-up in those studies was not long enough. I suppose if you follow long enough it is possible that all patients would progress to Alzheimer's disease but, nonetheless, the

NOVARTIS

T2000-11
89007401

Exelon®

(rivastigmine tartrate)

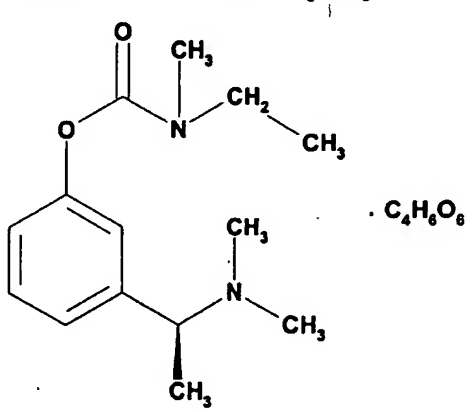
Capsules

Rx Only

Prescribing Information

DESCRIPTION

Exelon® (rivastigmine tartrate) is a reversible cholinesterase inhibitor and is known chemically as (S)-N-Ethyl-N-methyl-3-[1-(dimethylamino)ethyl]-phenyl carbamate hydrogen-(2R,3R)-tartrate. Rivastigmine tartrate is commonly referred to in the pharmacological literature as SDZ ENA 713 or ENA 713. It has an empirical formula of $C_{14}H_{22}N_2O_2 \cdot C_4H_6O_6$ (hydrogen tartrate salt – hta salt) and a molecular weight of 400.43 (hta salt). Rivastigmine tartrate is a white to off-white, fine crystalline powder that is very soluble in water, soluble in ethanol and acetonitrile, slightly soluble in n-octanol and very slightly soluble in ethyl acetate. The distribution coefficient at 37°C in n-octanol/phosphate buffer solution pH 7 is 3.0.



Exelon is supplied as capsules containing rivastigmine tartrate, equivalent to 1.5, 3.0, 4.5 and 6.0 mg of rivastigmine base for oral administration. Inactive ingredients are hydroxypropyl methylcellulose, magnesium stearate, microcrystalline cellulose, and silicone dioxide. Each hard-gelatin capsule contains gelatin, titanium dioxide and red and/or yellow iron oxides.

CLINICAL PHARMACOLOGY

Mechanism of Action

Pathological changes in Dementia of the Alzheimer type involve cholinergic neuronal pathways that project from the basal forebrain to the cerebral cortex and hippocampus. These pathways are thought to be intricately involved in memory, attention, learning, and other cognitive processes. While the precise mechanism of rivastigmine's action is unknown, it is postulated to exert its therapeutic effect by enhancing cholinergic function. This is accomplished by increasing the concentration of acetylcholine through reversible inhibition of its hydrolysis by cholinesterase. If this proposed mechanism is correct, Exelon's effect may lessen as the disease process advances and fewer cholinergic neurons remain functionally intact. There is no evidence that rivastigmine alters the course of the underlying dementing process. After a 6-mg

dose of rivastigmine, anticholinesterase activity is present in CSF for about 10 hours, with a maximum inhibition of about 60% five hours after dosing.

Clinical Trial Data

The effectiveness of Exelon® (rivastigmine tartrate) as a treatment for Alzheimer's Disease is demonstrated by the results of two randomized, double-blind, placebo-controlled clinical investigations in patients with Alzheimer's Disease [diagnosed by NINCDS-ADRDA and DSM-IV criteria, Mini-Mental State Examination (MMSE) ≥10 and ≤26, and the Global Deterioration Scale (GDS)]. The mean age of patients participating in Exelon trials was 73 years with a range of 41-95. Approximately 59% of patients were women and 41% were men. The racial distribution was Caucasian 87%, Black 4% and Other races 9%.

Study Outcome Measures: In each study, the effectiveness of Exelon was evaluated using a dual outcome assessment strategy.

The ability of Exelon to improve cognitive performance was assessed with the cognitive subscale of the Alzheimer's Disease Assessment Scale (ADAS-cog), a multi item instrument that has been extensively validated in longitudinal cohorts of Alzheimer's Disease patients. The ADAS-cog examines selected aspects of cognitive performance including elements of memory, orientation, attention, reasoning, language and praxis. The ADAS-cog scoring range is from 0 to 70, with higher scores indicating greater cognitive impairment. Elderly normal adults may score as low as 0 or 1, but it is not unusual for non-demented adults to score slightly higher.

The patients recruited as participants in each study had mean scores on ADAS-cog of approximately 23 units, with a range from 1 to 61. Experience gained in longitudinal studies of ambulatory patients with mild to moderate Alzheimer's Disease suggest that they gain 6-12 units a year on the ADAS-cog. Lesser degrees of change, however, are seen in patients with very mild or very advanced disease because the ADAS-cog is not uniformly sensitive to change over the course of the disease. The annualized rate of decline in the placebo patients participating in Exelon trials was approximately 3-8 units per year.

The ability of Exelon to produce an overall clinical effect was assessed using a Clinician's Interview Based Impression of Change that required the use of caregiver information, the CIBIC-Plus. The CIBIC-Plus is not a single instrument and is not a standardized instrument like the ADAS-cog. Clinical trials for investigational drugs have used a variety of CIBIC formats, each different in terms of depth and structure. As such, results from a CIBIC-Plus reflect clinical experience from the trial or trials in which it was used and can not be compared directly with the results of CIBIC-Plus evaluations from other clinical trials. The CIBIC-Plus used in the Exelon trials was a structured instrument based on a comprehensive evaluation at baseline and subsequent time-points of three domains: patient cognition, behavior and functioning, including assessment of activities of daily living. It represents the assessment of a skilled clinician using validated scales based on his/her observation at interviews conducted separately with the patient and the caregiver familiar with the behavior of the patient over the interval rated. The CIBIC-Plus is scored as a seven point categorical rating, ranging from a score of 1, indicating "markedly improved," to a score of 4, indicating "no change" to a score of 7, indicating "marked worsening." The CIBIC-Plus has not been systematically compared directly to assessments not using information from caregivers (CIBIC) or other global methods.

U.S. Twenty-Six-Week Study

In a study of 26 weeks duration, 699 patients were randomized to either a dose range of 1-4 mg or 6-12 mg of Exelon per day or to placebo, each given in divided doses. The 26-week study was divided into a

12-week forced dose titration phase and a 14-week maintenance phase. The patients in the active treatment arms of the study were maintained at their highest tolerated dose within the respective range.

Effects on the ADAS-cog: Figure 1 illustrates the time course for the change from baseline in ADAS-cog scores for all three dose groups over the 26 weeks of the study. At 26 weeks of treatment, the mean differences in the ADAS-cog change scores for the Exelon-treated patients compared to the patients on placebo were 1.9 and 4.9 units for the 1-4 mg and 6-12 mg treatments, respectively. Both treatments were statistically significantly superior to placebo and the 6-12 mg/day range was significantly superior to the 1-4 mg/day range.

Figure 1: Time-course of the Change from Baseline in ADAS-cog Score for Patients Completing 26 Weeks of Treatment

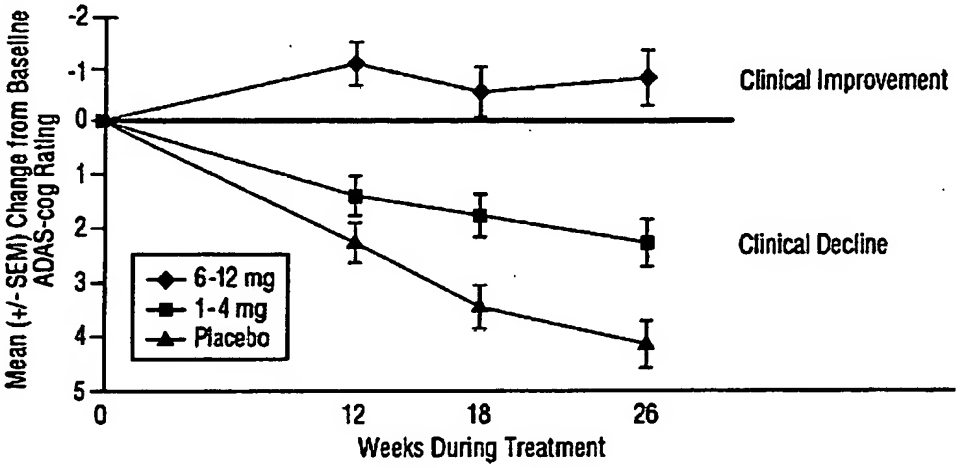
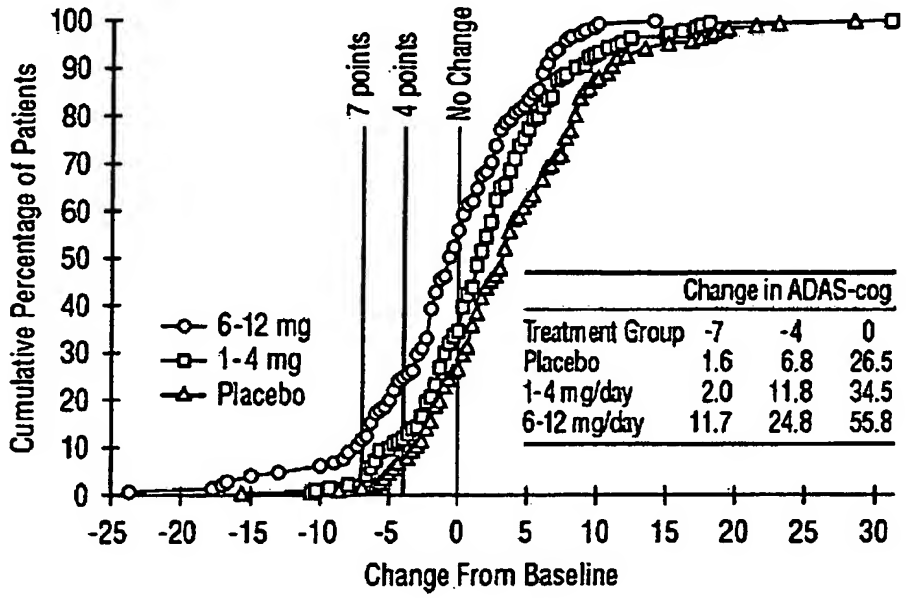


Figure 2 illustrates the cumulative percentages of patients from each of the three treatment groups who had attained at least the measure of improvement in ADAS-cog score shown on the X axis. Three change scores, (7-point and 4-point reductions from baseline or no change in score) have been identified for illustrative purposes, and the percent of patients in each group achieving that result is shown in the inset table.

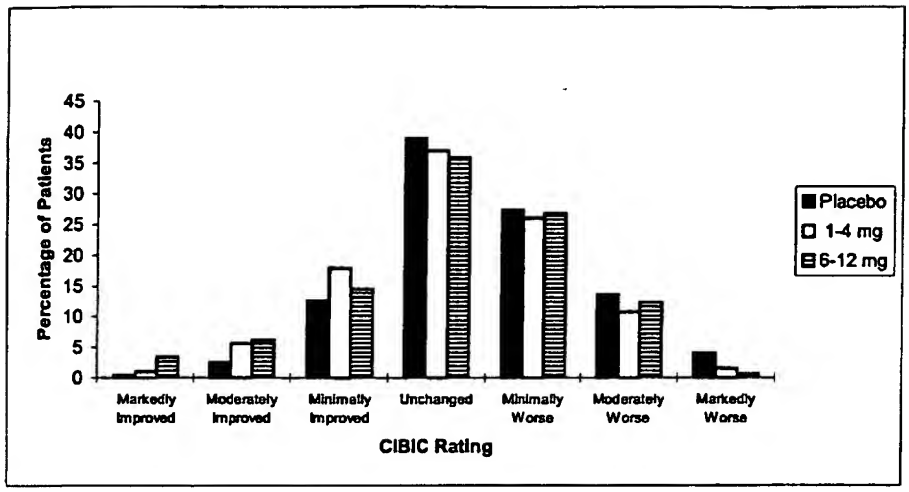
The curves demonstrate that both patients assigned to Exelon and placebo have a wide range of responses, but that the Exelon groups are more likely to show the greater improvements. A curve for an effective treatment would be shifted to the left of the curve for placebo, while an ineffective or deleterious treatment would be superimposed upon, or shifted to the right of the curve for placebo, respectively.

Figure 2: Cumulative Percentage of Patients Completing 26 Weeks of Double-blind Treatment with Specified Changes from Baseline ADAS-cog Scores. The Percentages of Randomized Patients who Completed the Study were: Placebo 84%, 1-4 mg 85%, and 6-12 mg 65%.



Effects on the CIBIC-Plus: Figure 3 is a histogram of the frequency distribution of CIBIC-Plus scores attained by patients assigned to each of the three treatment groups who completed 26 weeks of treatment. The mean Exelon-placebo differences for these groups of patients in the mean rating of change from baseline were 0.32 units and 0.35 units for 1-4 mg and 6-12 mg of Exelon, respectively. The mean ratings for the 6-12 mg/day and 1-4 mg/day groups were statistically significantly superior to placebo. The differences between the 6-12 mg/day and the 1-4 mg/day groups were statistically significant.

Figure 3: Frequency Distribution of CIBIC-Plus Scores at Week 26



Global Twenty-Six-Week Study

In a second study of 26 weeks duration, 725 patients were randomized to either a dose range of 1-4 mg or 6-12 mg of Exelon per day or to placebo, each given in divided doses. The 26-week study was divided into a 12-week forced dose titration phase and a 14-week maintenance phase. The patients in the active treatment arms of the study were maintained at their highest tolerated dose within the respective range.

Effects on the ADAS-cog: Figure 4 illustrates the time course for the change from baseline in ADAS-cog scores for all three dose groups over the 26 weeks of the study. At 26 weeks of treatment, the mean differences in the ADAS-cog change scores for the Exelon-treated patients compared to the patients on placebo were 0.2 and 2.6 units for the 1-4 mg and 6-12 mg treatments, respectively. The 6-12 mg/day group was statistically significantly superior to placebo, as well as to the 1-4 mg/day group. The difference between the 1-4 mg/day group and placebo was not statistically significant.

Figure 4: Time-course of the Change from Baseline in ADAS-cog Score for Patients Completing 26 Weeks of Treatment

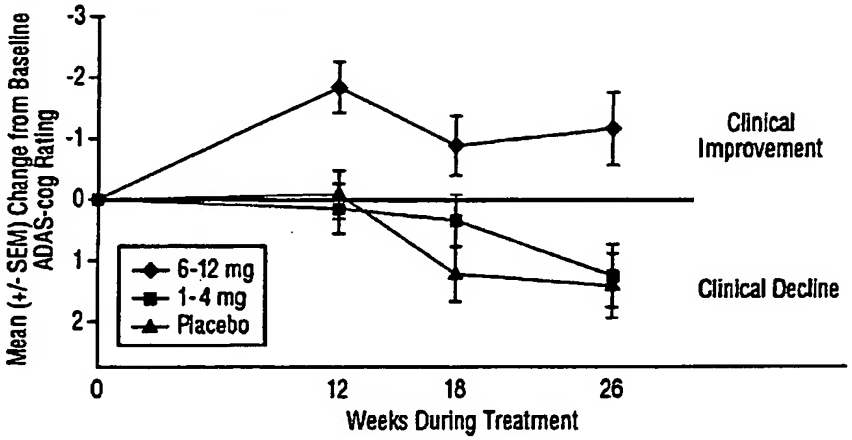
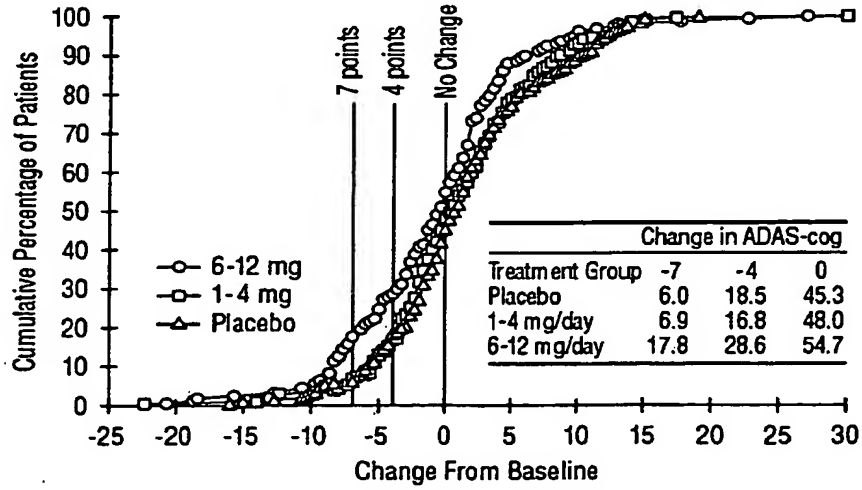


Figure 5 illustrates the cumulative percentages of patients from each of the three treatment groups who had attained at least the measure of improvement in ADAS-cog score shown on the X axis. Similar to the U.S. 26-week study, the curves demonstrate that both patients assigned to Exelon and placebo have a wide range of responses, but that the 6-12 mg/day Exelon group is more likely to show the greater improvements.

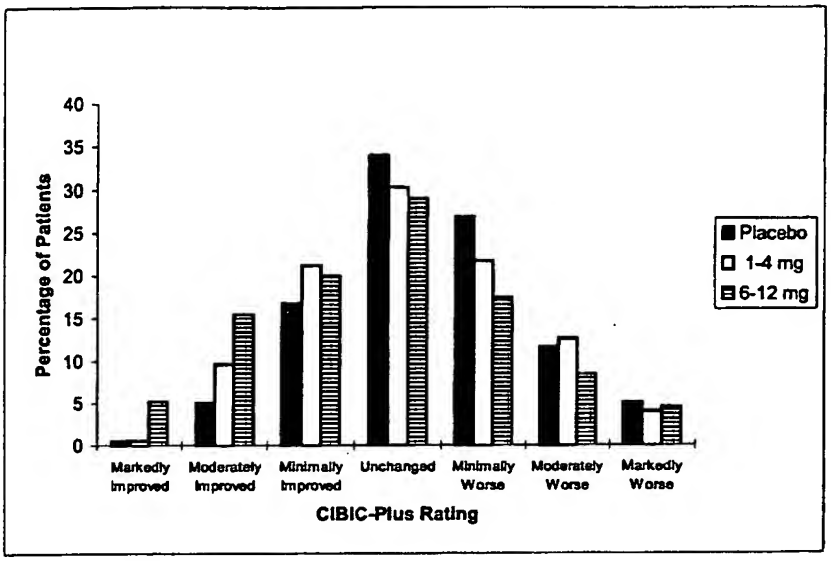
Figure 5: Cumulative Percentage of Patients Completing 26 Weeks of Double-blind Treatment with Specified Changes from Baseline ADAS-cog Scores. The Percentages of Randomized Patients who Completed the Study were: Placebo 87%, 1-4 mg 86%, and 6-12 mg 67%.



	Change in ADAS-cog		
Treatment Group	-7	-4	0
Placebo	6.0	18.5	45.3
1-4 mg/day	6.9	16.8	48.0
6-12 mg/day	17.8	28.6	54.7

Effects on the CIBIC-Plus: Figure 6 is a histogram of the frequency distribution of CIBIC-Plus scores attained by patients assigned to each of the three treatment groups who completed 26 weeks of treatment. The mean Exelon-placebo differences for these groups of patients for the mean rating of change from baseline were 0.14 units and 0.41 units for 1-4 mg and 6-12 mg of Exelon, respectively. The mean ratings for the 6-12 mg/day group was statistically significantly superior to placebo. The comparison of the mean ratings for the 1-4 mg/day group and placebo group was not statistically significant.

Figure 6: Frequency Distribution of CIBIC-Plus Scores at Week 26



U.S. Fixed Dose Study

In a study of 26 weeks' duration, 702 patients were randomized to doses of 3, 6, or 9 mg/day of Exelon or to placebo, each given in divided doses. The fixed-dose study design, which included a 12-week forced titration phase and a 14-week maintenance phase, led to a high dropout rate in the 9 mg/day group because of poor tolerability. At 26 weeks of treatment, significant differences were observed for the ADAS-cog mean change from baseline for the 9 mg/day and 6 mg/day groups, compared to placebo. No significant differences were observed between any of the Exelon dose groups and placebo for the analysis of the CIBIC-Plus mean rating of change. Although no significant differences were observed between Exelon treatment groups, there was a trend toward numerical superiority with higher doses.

Age, Gender and Race: Patient's age, gender, or race did not predict clinical outcome to Exelon treatment.

Pharmacokinetics

Rivastigmine is well absorbed with absolute bioavailability of about 40% (3-mg dose). It shows linear pharmacokinetics up to 3 mg BID but is non-linear at higher doses. Doubling the dose from 3 to 6 mg BID results in a 3-fold increase in AUC. The elimination half-life is about 1.5 hours, with most elimination as metabolites via the urine.

Absorption: Rivastigmine is rapidly and completely absorbed. Peak plasma concentrations are reached in approximately 1 hour. Absolute bioavailability after a 3-mg dose is about 36%. Administration of Exelon with food delays absorption (t_{max}) by 90 min, lowers C_{max} by approximately 30% and increases AUC by approximately 30%.

Distribution: Rivastigmine is widely distributed throughout the body with a volume of distribution in the range of 1.8-2.7 L/kg. Rivastigmine penetrates the blood brain barrier, reaching CSF peak concentrations in 1.4-2.6 hours. Mean $AUC_{1\text{-}12hr}$ ratio of CSF/plasma averaged 40 ± 0.5% following 1-6 mg BID doses.

Rivastigmine is about 40% bound to plasma proteins at concentrations of 1-400 ng/mL, which cover the therapeutic concentration range. Rivastigmine distributes equally between blood and plasma with a blood-to-plasma partition ratio of 0.9 at concentrations ranging from 1-400 ng/mL.

Metabolism: Rivastigmine is rapidly and extensively metabolized, primarily via cholinesterase-mediated hydrolysis to the decarbamylated metabolite. Based on evidence from *in vitro* and animal studies the major cytochrome P450 isozymes are minimally involved in rivastigmine metabolism. Consistent with these observations is the finding that no drug interactions related to cytochrome P450 have been observed in humans [see Drug-Drug Interactions].

Elimination: The major pathway of elimination is via the kidneys. Following administration of ^{14}C-rivastigmine to 6 healthy volunteers total recovery of radioactivity over 120 hours was 97% in urine and 0.4% in feces. No parent drug was detected in urine. The sulfate conjugate of the decarbamylated metabolite is the major component excreted in urine and represents 40% of the dose. Mean oral clearance of rivastigmine is 1.8 ± 0.6 L/min after 6 mg BID.

Special Populations

Hepatic Disease: Following a single 3-mg dose, mean oral clearance of rivastigmine was 60% lower in hepatically impaired patients (n=10, biopsy proven) than in healthy subjects (n=10). After multiple 6 mg BID oral dosing, the mean clearance of rivastigmine was 65% lower in mild (n=7, Child-Pugh score 5-6) and moderate (n=3, Child-Pugh score 7-9) hepatically impaired patients (biopsy proven, liver cirrhosis) than in healthy subjects (n=10). Dosage adjustment is not necessary in hepatically impaired patients as the dose of drug is individually titrated to tolerability.

Renal Disease: Following a single 3-mg dose, mean oral clearance of rivastigmine is 64% lower in moderately impaired renal patients (n=8, GFR=10-50 mL/min) than in healthy subjects (n=10, GFR≥60 mL/min); Cl/F=1.7 L/min (cv=45%) and 4.8 L/min (cv=80%), respectively. In severely impaired renal patients (n=8, GFR<10mL/min), mean oral clearance of rivastigmine is 43% higher than in healthy subjects (n=10, GFR≥60 mL/min); Cl/F = 6.9 L/min and 4.8 L/min, respectively. For unexplained reasons, the severely impaired renal patients had a higher clearance of rivastigmine than moderately impaired patients. However, dosage adjustment may not be necessary in renally impaired patients as the dose of the drug is individually titrated to tolerability.

Age: Following a single 2.5 mg oral dose to elderly volunteers (>60 years of age, n=24) and younger volunteers (n=24), mean oral clearance of rivastigmine was 30% lower in elderly (7 L/min) than in younger subjects (10 L/min).

Gender and Race: No specific pharmacokinetic study was conducted to investigate the effect of gender and race on the disposition of Exelon, but a population pharmacokinetic analysis indicates that gender (n=277 males and 348 females) and race (n=575 white, 34 black, 4 Asian, and 12 other) did not affect the clearance of Exelon.

Nicotine Use: Population PK analysis showed that nicotine use increases the oral clearance of rivastigmine by 23% (n=75 Smokers and 549 Nonsmokers).

Drug-Drug Interactions

Effect of Exelon on the Metabolism of Other Drugs: Rivastigmine is primarily metabolized through hydrolysis by esterases. Minimal metabolism occurs via the major cytochrome P450 isoenzymes. Based on *in vitro* studies, no pharmacokinetic drug interactions with drugs metabolized by the following isoenzyme systems are expected: CYP1A2, CYP2D6, CYP3A4/5, CYP2E1, CYP2C9, CYP2C8, or CYP2C19.

No pharmacokinetic interaction was observed between rivastigmine and digoxin, warfarin, diazepam, or fluoxetine in studies in healthy volunteers. The elevation of prothrombin time induced by warfarin is not affected by administration of Exelon.

Effect of Other Drugs on the Metabolism of Exelon: Drugs that induce or inhibit CYP450 metabolism are not expected to alter the metabolism of rivastigmine. Single dose pharmacokinetic studies demonstrated that the metabolism of rivastigmine is not significantly affected by concurrent administration of digoxin, warfarin, diazepam, or fluoxetine.

Population PK analysis with a database of 625 patients showed that the pharmacokinetics of rivastigmine were not influenced by commonly prescribed medications such as antacids (n=77), antihypertensives (n=72), β-blockers (n=42), calcium channel blockers (n=75), antidiabetics (n=21), non-steroidal anti-inflammatory drugs (n=79), estrogens (n=70), salicylate analgesics (n=177), antianginals (n=35), and antihistamines (n=15). In addition, in clinical trials, no increased risk of clinically relevant untoward effects was observed in patients treated concomitantly with Exelon and these agents.

INDICATIONS AND USAGE

Exelon® (rivastigmine tartrate) is indicated for the treatment of mild to moderate dementia of the Alzheimer's type.

CONTRAINDICATIONS

Exelon® (rivastigmine tartrate) is contraindicated in patients with known hypersensitivity to rivastigmine, other carbamate derivatives or other components of the formulation (see DESCRIPTION).

WARNINGS

Gastrointestinal Adverse Reactions

Exelon® (rivastigmine tartrate) use is associated with significant gastrointestinal adverse reactions, including nausea and vomiting, anorexia, and weight loss. For this reason, patients should always be started at a dose of 1.5 mg BID and titrated to their maintenance dose. If treatment is interrupted for longer than several days, treatment should be reinitiated with the lowest daily dose (see Dosage and Administration) to reduce the possibility of severe vomiting and its potentially serious sequelae (e.g., there has been one post-marketing report of severe vomiting with esophageal rupture following inappropriate reinitiation of treatment with a 4.5 mg dose after 8 weeks of treatment interruption.)

Nausea and Vomiting: **In the controlled clinical trials, 47% of the patients treated with an Exelon dose in the therapeutic range of 6-12 mg/day (n=1189) developed nausea (compared with 12% in placebo). A total of 31% of Exelon-treated patients developed at least one episode of vomiting (compared with 6% for placebo). The rate of vomiting was higher during the titration phase (24% vs. 3% for placebo) than in the maintenance phase (14% vs. 3% for placebo). The rates were higher in women than men. Five percent of patients discontinued for vomiting, compared to less than 1% for patients on placebo. Vomiting was severe in 2% of Exelon-treated patients and was rated as mild or moderate each in 14% of patients. The rate of nausea was higher during the titration phase (43% vs. 9% for placebo) than in the maintenance phase (17% vs. 4% for placebo).**

Weight Loss: **In the controlled trials, approximately 26% of women on high doses of Exelon (greater than 9 mg/day) had weight loss of equal to or greater than 7% of their baseline weight compared to 6% in the placebo-treated patients. About 18% of the males in the high dose group experienced a similar degree of weight loss compared to 4% in placebo-treated patients. It is not**

clear how much of the weight loss was associated with anorexia, nausea, vomiting, and the diarrhea associated with the drug.

Anorexia: In the controlled clinical trials, of the patients treated with an Exelon dose of 6-12 mg/day, 17% developed anorexia compared to 3% of the placebo patients. Neither the time course or the severity of the anorexia is known.

Peptic Ulcers/Gastrointestinal Bleeding: Because of their pharmacological action, cholinesterase inhibitors may be expected to increase gastric acid secretion due to increased cholinergic activity. Therefore, patients should be monitored closely for symptoms of active or occult gastrointestinal bleeding, especially those at increased risk for developing ulcers, e.g., those with a history of ulcer disease or those receiving concurrent nonsteroidal anti-inflammatory drugs (NSAIDS). Clinical studies of Exelon have shown no significant increase, relative to placebo, in the incidence of either peptic ulcer disease or gastrointestinal bleeding.

Anesthesia

Exelon as a cholinesterase inhibitor, is likely to exaggerate succinylcholine-type muscle relaxation during anesthesia.

Cardiovascular Conditions

Drugs that increase cholinergic activity may have vagotonic effects on heart rate (e.g., bradycardia). The potential for this action may be particularly important to patients with "sick sinus syndrome" or other supraventricular cardiac conduction conditions. In clinical trials, Exelon was not associated with any increased incidence of cardiovascular adverse events, heart rate or blood pressure changes, or ECG abnormalities. Syncopal episodes have been reported in 3% of patients receiving 6–12 mg/day of Exelon, compared to 2% of placebo patients.

Genitourinary

Although this was not observed in clinical trials of Exelon, drugs that increase cholinergic activity may cause urinary obstruction.

Neurological Conditions

Seizures: Drugs that increase cholinergic activity are believed to have some potential for causing seizures. However, seizure activity also may be a manifestation of Alzheimer's Disease.

Pulmonary Conditions

Like other drugs that increase cholinergic activity, Exelon should be used with care in patients with a history of asthma or obstructive pulmonary disease.

PRECAUTIONS

Information for Patients and Caregivers

Caregivers should be advised of the high incidence of nausea and vomiting associated with the use of the drug along with the possibility of anorexia and weight loss. Caregivers should be encouraged to monitor for these adverse events and inform the physician if they occur. It is critical to inform caregivers that if therapy has been interrupted for more than several days, the next dose should not be administered until they have discussed this with the physician.

Drug-Drug Interactions

Effect of Exelon® (rivastigmine tartrate) on the Metabolism of Other Drugs: Rivastigmine is primarily metabolized through hydrolysis by esterases. Minimal metabolism occurs via the major cytochrome P450 isoenzymes. Based on *in vitro* studies, no pharmacokinetic drug interactions with drugs metabolized by the following isoenzyme systems are expected: CYP1A2, CYP2D6, CYP3A4/5, CYP2E1, CYP2C9, CYP2C8, or CYP2C19.

No pharmacokinetic interaction was observed between rivastigmine and digoxin, warfarin, diazepam, or fluoxetine in studies in healthy volunteers. The elevation of prothrombin time induced by warfarin is not affected by administration of Exelon.

Effect of Other Drugs on the Metabolism of Exelon: Drugs that induce or inhibit CYP450 metabolism are not expected to alter the metabolism of rivastigmine. Single dose pharmacokinetic studies demonstrated that the metabolism of rivastigmine is not significantly affected by concurrent administration of digoxin, warfarin, diazepam, or fluoxetine.

Population PK analysis with a database of 625 patients showed that the pharmacokinetics of rivastigmine were not influenced by commonly prescribed medications such as antacids (n=77), antihypertensives (n=72), β-blockers (n=42), calcium channel blockers (n=75), antidiabetics (n=21), nonsteroidal anti-inflammatory drugs (n=79), estrogens (n=70), salicylate analgesics (n=177), antianginals (n=35), and antihistamines (n=15).

Use with Anticholinergics: Because of their mechanism of action, cholinesterase inhibitors have the potential to interfere with the activity of anticholinergic medications.

Use with Cholinomimetics and Other Cholinesterase Inhibitors: A synergistic effect may be expected when cholinesterase inhibitors are given concurrently with succinylcholine, similar neuromuscular blocking agents or cholinergic agonists such as bethanechol.

Carcinogenesis, Mutagenesis, Impairment of Fertility

In carcinogenicity studies conducted at dose levels up to 1.1 mg-base/kg/day in rats and 1.6 mg-base/kg/day in mice, rivastigmine was not carcinogenic. These dose levels are approximately 0.9 times and 0.7 times the maximum recommended human daily dose of 12 mg per day on a mg/m^2 basis.

Rivastigmine was clastogenic in two *in vitro* assays in the presence, but not the absence, of metabolic activation. It caused structural chromosomal aberrations in V79 Chinese hamster lung cells and both structural and numerical (polyploidy) chromosomal aberrations in human peripheral blood lymphocytes. Rivastigmine was not genotoxic in three *in vitro* assays: the Ames test, the unscheduled DNA synthesis (UDS) test in rat hepatocytes (a test for induction of DNA repair synthesis), and the HGPRT test in V79 Chinese hamster cells. Rivastigmine was not clastogenic in the *in vivo* mouse micronucleus test.

Rivastigmine had no effect on fertility or reproductive performance in the rat at dose levels up to 1.1 mg-base/kg/day. This dose is approximately 0.9 times the maximum recommended human daily dose of 12 mg/per day on a mg/m^2 basis.

Pregnancy

Pregnancy Category B: Reproduction studies conducted in pregnant rats at doses up to 2.3 mg-base/kg/day (approximately 2 times the maximum recommended human dose on a mg/m^2 basis) and in pregnant rabbits at doses up to 2.3 mg-base/kg/day (approximately 4 times the maximum recommended human dose on a mg/m^2 basis) revealed no evidence of teratogenicity. Studies in rats showed slightly decreased fetal/pup

weights, usually at doses causing some maternal toxicity; decreased weights were seen at doses which were several fold lower than the maximum recommended human dose on a mg/m^2 basis. There are no adequate or well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, Exelon should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nursing Mothers

It is not known whether rivastigmine is excreted in human breast milk. Exelon has no indication for use in nursing mothers.

Pediatric Use

There are no adequate and well-controlled trials documenting the safety and efficacy of Exelon in any illness occurring in children.

ADVERSE REACTIONS

Adverse Events Leading to Discontinuation

The rate of discontinuation due to adverse events in controlled clinical trials of Exelon® (rivastigmine tartrate) was 15% for patients receiving 6-12 mg/day compared to 5% for patients on placebo during forced weekly dose titration. While on a maintenance dose, the rates were 6% for patients on Exelon compared to 4% for those on placebo.

The most common adverse events leading to discontinuation, defined as those occurring in at least 2% of patients and at twice the incidence seen in placebo patients, are shown in Table 1.

Table 1. Most Frequent Adverse Events Leading to Withdrawal from Clinical Trials during Titration and Maintenance in Patients Receiving 6-12 mg/day Exelon® Using a Forced Dose Titration

Study Phase	Titration		Maintenance		Overall	
	Placebo (n=868)	Exelon ≥6-12 mg/day (n=1189)	Placebo (n=788)	Exelon ≥6-12 mg/day (n=987)	Placebo (n=868)	Exelon ≥6-12 mg/day (n=1189)
Event/% Discontinuing						
Nausea	<1	8	<1	1	1	8
Vomiting	<1	4	<1	1	<1	5
Anorexia	0	2	<1	1	<1	3
Dizziness	<1	2	<1	1	<1	2

Most Frequent Adverse Clinical Events Seen in Association with the Use of Exelon

The most common adverse events, defined as those occurring at a frequency of at least 5% and twice the placebo rate, are largely predicted by Exelon's cholinergic effects. These include nausea, vomiting, anorexia, dyspepsia, and asthenia.

Gastrointestinal Adverse Reactions

Exelon use is associated with significant nausea, vomiting, and weight loss (see WARNINGS).

Adverse Events Reported in Controlled Trials

Table 2 lists treatment emergent signs and symptoms that were reported in at least 2% of patients in placebo-controlled trials and for which the rate of occurrence was greater for patients treated with Exelon doses of 6-12 mg/day than for those treated with placebo. The prescriber should be aware that these figures cannot be used to predict the frequency of adverse events in the course of usual medical practice when patient characteristics and other factors may differ from those prevailing during clinical studies. Similarly, the cited frequencies cannot be directly compared with figures obtained from other clinical investigations involving different treatments, uses, or investigators. An inspection of these frequencies, however, does provide the prescriber with one basis by which to estimate the relative contribution of drug and non-drug factors to the adverse event incidences in the population studied.

In general, adverse reactions were less frequent later in the course of treatment.

No systematic effect of race or age could be determined on the incidence of adverse events in the controlled studies. Nausea, vomiting and weight loss were more frequent in women than men.

Table 2. Adverse Events Reported in Controlled Clinical Trials in at Least 2% of Patients Receiving Exelon® (6-12 mg/day) and at a Higher Frequency than Placebo-treated Patients

Body System/Adverse Event	Placebo (n=868)	Exelon® (6-12 mg/day) (n=1189)
Percent of Patients with any Adverse Event	79	92
Autonomic Nervous System		
Sweating Increased	1	4
Syncope	2	3
Body as a Whole		
Accidental Trauma	9	10
Fatigue	5	9
Asthenia	2	6
Malaise	2	5
Influenza-like Symptoms	2	3
Weight Decrease	<1	3
Cardiovascular Disorders, General		
Hypertension	2	3
Central and Peripheral Nervous System		
Dizziness	11	21
Headache	12	17
Somnolence	3	5
Tremor	1	4
Gastrointestinal System		
Nausea	12	47
Vomiting	6	31
Diarrhea	11	19
Anorexia	3	17
Abdominal Pain	6	13
Dyspepsia	4	9
Constipation	4	5
Flatulence	2	4
Eructation	1	2
Psychiatric Disorders		
Insomnia	7	9
Confusion	7	8
Depression	4	6
Anxiety	3	5
Hallucination	3	4
Aggressive Reaction	2	3
Resistance Mechanism Disorders		
Urinary Tract Infection	6	7
Respiratory System		
Rhinitis	3	4

Other adverse events observed at a rate of 2% or more on Exelon 6-12 mg/day but at a greater or equal rate on placebo were chest pain, peripheral edema, vertigo, back pain, arthralgia, pain, bone fracture, agitation, nervousness, delusion, paranoid reaction, upper respiratory tract infections, infection (general), coughing, pharyngitis, bronchitis, rash (general), urinary incontinence.

Other Adverse Events Observed During Clinical Trials

Exelon has been administered to over 5297 individuals during clinical trials worldwide. Of these, 4326 patients have been treated for at least 3 months, 3407 patients have been treated for at least 6 months, 2150 patients have been treated for 1 year, 1250 have been treated for 2 years, and 168 have been treated for over 3 years. With regard to exposure to the highest dose, 2809 patients were exposed to doses of 10-12 mg, 2615 patients treated for 3 months, 2328 patients treated for 6 months, 1378 patients treated for 1 year, 917 patients treated for 2 years, and 129 treated for over 3 years.

Treatment emergent signs and symptoms that occurred during 8 controlled clinical trials and 9 open-label trials in North America, Western Europe, Australia, South Africa, and Japan were recorded as adverse events by the clinical investigators using terminology of their own choosing. To provide an overall estimate of the proportion of individuals having similar types of events, the events were grouped into a smaller number of standardized categories using a modified WHO dictionary, and event frequencies were calculated across all studies. These categories are used in the listing below. The frequencies represent the proportion of 5297 patients from these trials who experienced that event while receiving Exelon. All adverse events occurring in at least 6 patients (approximately 0.1%) are included, except for those already listed elsewhere in labeling, WHO terms too general to be informative, relatively minor events, or events unlikely to be drug caused. Events are classified by body system and listed using the following definitions: frequent adverse events - those occurring in at least 1/100 patients; infrequent adverse events - those occurring in 1/100 to 1/1000 patients. These adverse events are not necessarily related to Exelon treatment and in most cases were observed at a similar frequency in placebo-treated patients in the controlled studies.

Autonomic Nervous System: *Infrequent:* Cold clammy skin, dry mouth, flushing, increased saliva.

Body as a Whole: *Frequent:* Accidental trauma, fever, edema, allergy, hot flushes, rigors. *Infrequent*: Edema periorbital or facial, hypothermia, edema, feeling cold, halitosis.

Cardiovascular System: *Frequent:* Hypotension, postural hypotension, cardiac failure.

Central and Peripheral Nervous System: *Frequent:* Abnormal gait, ataxia, paraesthesia, convulsions. *Infrequent:* Paresis, apraxia, aphasia, dysphonia, hyperkinesia, hyperreflexia, hypertonia, hypoesthesia, hypokinesia, migraine, neuralgia, nystagmus, peripheral neuropathy.

Endocrine System: *Infrequent:* Goitre, hypothyroidism.

Gastrointestinal System: *Frequent:* Fecal incontinence, gastritis. *Infrequent:* Dysphagia, esophagitis, gastric ulcer, gastritis, gastroesophageal reflux, GI hemorrhage, hernia, intestinal obstruction, melena, rectal hemorrhage, gastroenteritis, ulcerative stomatitis, duodenal ulcer, hematemesis, gingivitis, tenesmus, pancreatitis, colitis, glossitis.

Hearing and Vestibular Disorders: *Frequent:* Tinnitus.

Heart Rate and Rhythm Disorders: *Frequent:* Atrial fibrillation, bradycardia, palpitation. *Infrequent:* AV block, bundle branch block, sick sinus syndrome, cardiac arrest, supraventricular tachycardia, extrasystoles, tachycardia.

Liver and Biliary System Disorders: *Infrequent:* Abnormal hepatic function, cholecystitis.

Metabolic and Nutritional Disorders: *Frequent:* Dehydration, hypokalemia. *Infrequent:* Diabetes mellitus, gout, hypercholesterolemia, hyperlipemia, hypoglycemia, cachexia, thirst, hyperglycemia, hyponatremia.

Musculoskeletal Disorders: *Frequent:* Arthritis, leg cramps, myalgia. *Infrequent:* Cramps, hernia, muscle weakness.

Myo-, Endo-, Pericardial and Valve Disorders: *Frequent:* Angina pectoris, myocardial infarction.

Platelet, Bleeding, and Clotting Disorders: *Frequent:* Epistaxis. *Infrequent:* Hematoma, thrombocytopenia, purpura.

Psychiatric Disorders: *Frequent:* Paranoid reaction, confusion. *Infrequent:* Abnormal dreaming, amnesia, apathy, delirium, dementia, depersonalization, emotional lability, impaired concentration,

decreased libido, personality disorder, suicide attempt, increased libido, neurosis, suicidal ideation, psychosis.

Red Blood Cell Disorders: *Frequent:* Anemia. *Infrequent:* Hypochromic anemia.

Reproductive Disorders (Female & Male): *Infrequent:* Breast pain, impotence, atrophic vaginitis.

Resistance Mechanism Disorders: *Infrequent:* Cellulitis, cystitis, herpes simplex, otitis media.

Respiratory System: *Infrequent:* Bronchospasm, laryngitis, apnea.

Skin and Appendages: *Frequent:* Rashes of various kinds (maculopapular, eczema, bullous, exfoliative, psoriaform, erythematous). *Infrequent:* Alopecia, skin ulceration, urticaria, dermatitis contact.

Special Senses: *Infrequent:* Perversion of taste, loss of taste.

Urinary System Disorders: *Frequent:* Hematuria. *Infrequent:* Albuminuria, oliguria, acute renal failure, dysuria, micturition urgency, nocturia, polyuria, renal calculus, urinary retention.

Vascular (extracardiac) Disorders: *Infrequent:* Hemorrhoids, peripheral ischemia, pulmonary embolism, thrombosis, thrombophlebitis deep, aneurysm, hemorrhage intracranial.

Vision Disorders: *Frequent:* Cataract. *Infrequent:* Conjunctival hemorrhage, blepharitis, diplopia, eye pain, glaucoma.

White Cell and Resistance Disorders: *Infrequent:* Lymphadenopathy, leukocytosis.

Post-Introduction Reports

Voluntary reports of adverse events temporally associated with Exelon that have been received since market introduction that are not listed above, and that may or may not be causally related to the drug include the following:

Skin and Appendages: Stevens-Johnson syndrome

OVERDOSAGE

Because strategies for the management of overdose are continually evolving, it is advisable to contact a Poison Control Center to determine the latest recommendations for the management of an overdose of any drug.

As Exelon® (rivastigmine tartrate) has a short plasma half-life of about one hour and a moderate duration of acetylcholinesterase inhibition of 8-10 hours, it is recommended that in cases of asymptomatic overdoses, no further dose of Exelon should be administered for the next 24 hours.

As in any case of overdose, general supportive measures should be utilized. Overdosage with cholinesterase inhibitors can result in cholinergic crisis characterized by severe nausea, vomiting, salivation, sweating, bradycardia, hypotension, respiratory depression, collapse and convulsions. Increasing muscle weakness is a possibility and may result in death if respiratory muscles are involved. Atypical responses in blood pressure and heart rate have been reported with other drugs that increase cholinergic activity when co-administered with quaternary anticholinergics such as glycopyrrolate. Due to the short half-life of Exelon, dialysis (hemodialysis, peritoneal dialysis, or hemofiltration) would not be clinically indicated in the event of an overdose.

In overdoses accompanied by severe nausea and vomiting, the use of antiemetics should be considered. In a documented case of a 46 mg overdose with Exelon, the patient experienced vomiting,

incontinence, hypertension, psychomotor retardation, and loss of consciousness. The patient fully recovered within 24 hours and conservative management was all that was required for treatment.

DOSAGE AND ADMINISTRATION

The dosage of Exelon® (rivastigmine tartrate) shown to be effective in controlled clinical trials is 6-12 mg/day, given as twice a day dosing (daily doses of 3 to 6 mg BID). There is evidence from the clinical trials that doses at the higher end of this range may be more beneficial.

The starting dose of Exelon is 1.5 mg twice a day (BID). If this dose is well tolerated, after a minimum of two weeks of treatment, the dose may be increased to 3 mg BID. Subsequent increases to 4.5 mg BID and 6 mg BID should be attempted after a minimum of 2 weeks at the previous dose. If adverse effects (e.g., nausea, vomiting, abdominal pain, loss of appetite) cause intolerance during treatment, the patient should be instructed to discontinue treatment for several doses and then restart at the same or next lower dose level. If treatment is interrupted for longer than several days, treatment should be reinitiated with the lowest daily dose and titrated as described above (see Warnings). The maximum dose is 6 mg BID (12 mg/day).

Exelon should be taken with meals in divided doses in the morning and evening.

HOW SUPPLIED

Exelon® (rivastigmine tartrate) capsules equivalent to 1.5 mg, 3.0 mg, 4.5 mg, or 6.0 mg of rivastigmine base are available as follows:

1.5 mg Capsule – yellow, "Exelon 1,5 mg" is printed in red on the body of the capsule.

Bottles of 60 ..... NDC 0078-0323-44
Bottles of 500 ..... NDC 0078-0323-08
Unit Dose (blister pack)
Box of 100 (strips of 10) ..... NDC 0078-0323-06

3.0 mg Capsule – orange, "Exelon 3 mg" is printed in red on the body of the capsule.

Bottles of 60 ..... NDC 0078-0324-44
Bottles of 500 ..... NDC 0078-0324-08
Unit Dose (blister pack)
Box of 100 (strips of 10) ..... NDC 0078-0324-06

4.5 mg Capsule – red, "Exelon 4,5 mg" is printed in white on the body of the capsule.

Bottles of 60 ..... NDC 0078-0325-44
Bottles of 500 ..... NDC 0078-0325-08
Unit Dose (blister pack)
Box of 100 (strips of 10) ..... NDC 0078-0325-06

6.0 mg Capsule - orange and red, "Exelon 6 mg" is printed in red on the body of the capsule.

Bottles of 60 ..... NDC 0078-0326-44
Bottles of 500 ..... NDC 0078-0326-08
Unit Dose (blister pack)
Box of 100 (strips of 10) ..... NDC 0078-0326-06

Store below 77°F (25°C) in a tight container.

T2000-11
REV: DECEMBER 2000 Printed in U.S.A. 89007401

NOVARTIS

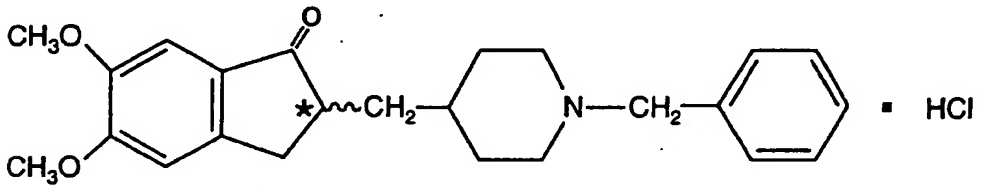
Manufactured by
Novartis Pharma AG
Basle, Switzerland

Manufactured for
Novartis Pharmaceuticals Corporation
East Hanover, New Jersey 07936

ARICEPT®Oral Solution
(Donepezil Hydrochloride)

DESCRIPTION

ARICEPT® (donepezil hydrochloride) is a reversible inhibitor of the enzyme acetylcholinesterase, known chemically as (±)-2,3-dihydro-5,6-dimethoxy-2-[[1-(phenylmethyl)-4-piperidinyl]methyl]-1*H*-inden-1-one hydrochloride. Donepezil hydrochloride is commonly referred to in the pharmacological literature as E2020. It has an empirical formula of $C_{24}H_{29}NO_3HCl$ and a molecular weight of 415.96. Donepezil hydrochloride is a white crystalline powder and is freely soluble in chloroform, soluble in water and in glacial acetic acid, slightly soluble in ethanol and in acetonitrile and practically insoluble in ethyl acetate and in n-hexane.



Each 1mL of ARICEPT® Oral Solution contains 1 mg of donepezil hydrochloride. ARICEPT® Oral Solution also contains sorbitol solution 70%, povidone K-30, citric acid anhydrous, sodium citrate dihydrate, sodium benzoate, methylparaben, propylene glycol, sodium metabisulfite, purified water and strawberry flavor.

CLINICAL PHARMACOLOGY

Current theories on the pathogenesis of the cognitive signs and symptoms of Alzheimer's Disease attribute some of them to a deficiency of cholinergic neurotransmission.

Donepezil hydrochloride is postulated to exert its therapeutic effect by enhancing cholinergic function. This is accomplished by increasing the concentration of acetylcholine through reversible inhibition of its hydrolysis by acetylcholinesterase. If this proposed mechanism of action is correct, donepezil's effect may lessen as the disease process advances and fewer cholinergic neurons remain functionally intact. There is no evidence that donepezil alters the course of the underlying dementing process.

Clinical Trial Data

The effectiveness of ARICEPT® as a treatment for Alzheimer's Disease is demonstrated by the results of two randomized, double-blind, placebo-controlled clinical investigations

in patients with Alzheimer's Disease (diagnosed by NINCDS and DSM III-R criteria, Mini-Mental State Examination ≥ 10 and ≤ 26 and Clinical Dementia Rating of 1 or 2). The mean age of patients participating in ARICEPT® trials was 73 years with a range of 50 to 94. Approximately 62% of patients were women and 38% were men. The racial distribution was white 95%, black 3% and other races 2%.

Study Outcome Measures: In each study, the effectiveness of treatment with ARICEPT® was evaluated using a dual outcome assessment strategy.

The ability of ARICEPT® to improve cognitive performance was assessed with the cognitive subscale of the Alzheimer's Disease Assessment Scale (ADAS-cog), a multi-item instrument that has been extensively validated in longitudinal cohorts of Alzheimer's Disease patients. The ADAS-cog examines selected aspects of cognitive performance including elements of memory, orientation, attention, reasoning, language and praxis. The ADAS-cog scoring range is from 0 to 70, with higher scores indicating greater cognitive impairment. Elderly normal adults may score as low as 0 or 1, but it is not unusual for non-demented adults to score slightly higher.

The patients recruited as participants in each study had mean scores on the Alzheimer's Disease Assessment Scale (ADAS-cog) of approximately 26 units, with a range from 4 to 61. Experience gained in longitudinal studies of ambulatory patients with mild to moderate Alzheimer's Disease suggest that they gain 6 to 12 units a year on the ADAS-cog. However, lesser degrees of change are seen in patients with very mild or very advanced disease because the ADAS-cog is not uniformly sensitive to change over the course of the disease. The annualized rate of decline in the placebo patients participating in ARICEPT® trials was approximately 2 to 4 units per year.

The ability of ARICEPT® to produce an overall clinical effect was assessed using a Clinician's Interview Based Impression of Change that required the use of caregiver information, the CIBIC plus. The CIBIC plus is not a single instrument and is not a standardized instrument like the ADAS-cog. Clinical trials for investigational drugs have used a variety of CIBIC formats, each different in terms of depth and structure.

As such, results from a CIBIC plus reflect clinical experience from the trial or trials in which it was used and cannot be compared directly with the results of CIBIC plus evaluations from other clinical trials. The CIBIC plus used in ARICEPT® trials was a semi-structured instrument that was intended to examine four major areas of patient function: General, Cognitive, Behavioral and Activities of Daily Living. It represents the assessment of a skilled clinician based upon his/her observations at an interview with the patient, in combination with information supplied by a caregiver familiar with the behavior of the patient over the interval rated. The CIBIC plus is scored as a seven point categorical rating, ranging from a score of 1, indicating "markedly improved," to a score of 4, indicating "no change" to a score of 7, indicating "markedly worse." The CIBIC plus has not been systematically compared directly to assessments not using information from caregivers (CIBIC) or other global methods.

Thirty-Week Study

In a study of 30 weeks duration, 473 patients were randomized to receive single daily doses of placebo, 5 mg/day or 10 mg/day of ARICEPT®. The 30-week study was divided into a 24-week double-blind active treatment phase followed by a 6-week single-blind placebo washout period. The study was designed to compare 5 mg/day or 10 mg/day fixed doses of ARICEPT® to placebo. However, to reduce the likelihood of cholinergic effects, the 10 mg/day treatment was started following an initial 7-day treatment with 5 mg/day doses.

Effects on the ADAS-cog: Figure 1 illustrates the time course for the change from baseline in ADAS-cog scores for all three dose groups over the 30 weeks of the study. After 24 weeks of treatment, the mean differences in the ADAS-cog change scores for ARICEPT® treated patients compared to the patients on placebo were 2.8 and 3.1 units for the 5 mg/day and 10 mg/day treatments, respectively. These differences were statistically significant. While the treatment effect size may appear to be slightly greater for the 10 mg/day treatment, there was no statistically significant difference between the two active treatments.

Following 6 weeks of placebo washout, scores on the ADAS-cog for both the ARICEPT® treatment groups were indistinguishable from those patients who had received only placebo for 30 weeks. This suggests that the beneficial effects of ARICEPT® abate over 6 weeks following discontinuation of treatment and do not represent a change in the underlying disease. There was no evidence of a rebound effect 6 weeks after abrupt discontinuation of therapy.

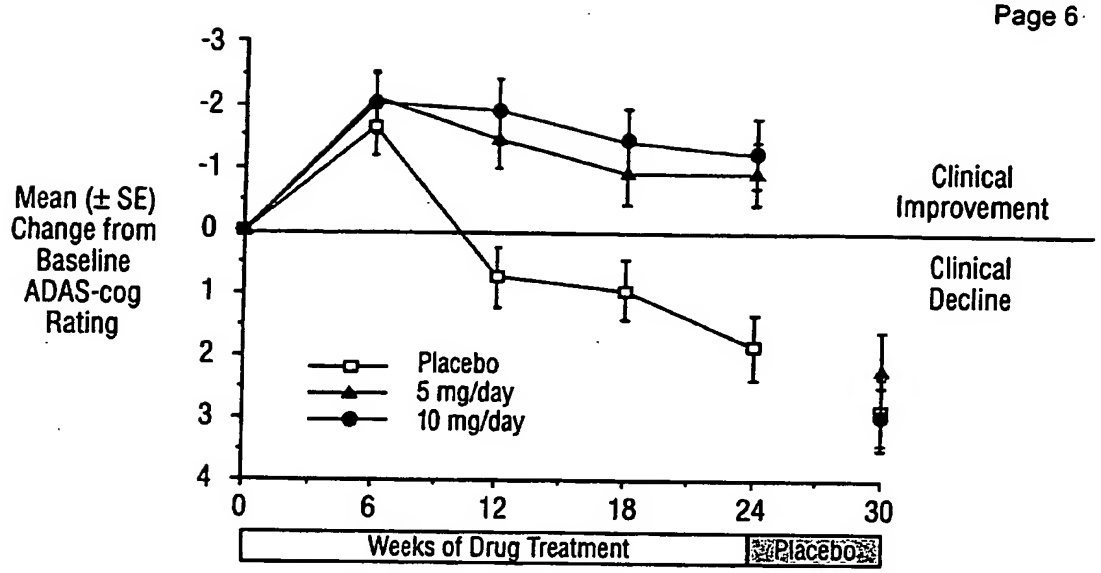


Figure 1. Time-course of the Change from Baseline in ADAS-cog Score for Patients Completing 24 Weeks of Treatment.

Figure 2 illustrates the cumulative percentages of patients from each of the three treatment groups who had attained the measure of improvement in ADAS-cog score shown on the X axis. Three change scores, (7-point and 4-point reductions from baseline or no change in score) have been identified for illustrative purposes and the percent of patients in each group achieving that result is shown in the inset table.

The curves demonstrate that both patients assigned to placebo and ARICEPT® have a wide range of responses, but that the active treatment groups are more likely to show the greater improvements. A curve for an effective treatment would be shifted to the left of the curve for placebo, while an ineffective or deleterious treatment would be superimposed upon or shifted to the right of the curve for placebo, respectively.

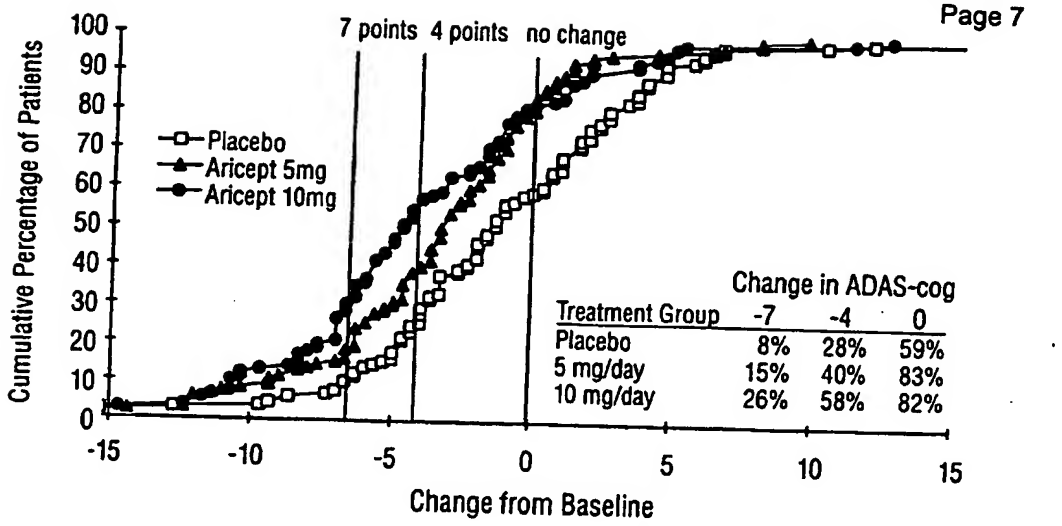


Figure 2. Cumulative Percentage of Patients Completing 24 Weeks of Double-blind Treatment with Specified Changes from Baseline ADAS-cog Scores. The Percentages of Randomized Patients who Completed the Study were: Placebo 80%, 5 mg/day 85% and 10 mg/day 68%.

Effects on the CIBIC plus: Figure 3 is a histogram of the frequency distribution of CIBIC plus scores attained by patients assigned to each of the three treatment groups who completed 24 weeks of treatment. The mean drug-placebo differences for these groups of patients were 0.35 units and 0.39 units for 5 mg/day and 10 mg/day of ARICEPT®, respectively. These differences were statistically significant. There was no statistically significant difference between the two active treatments.

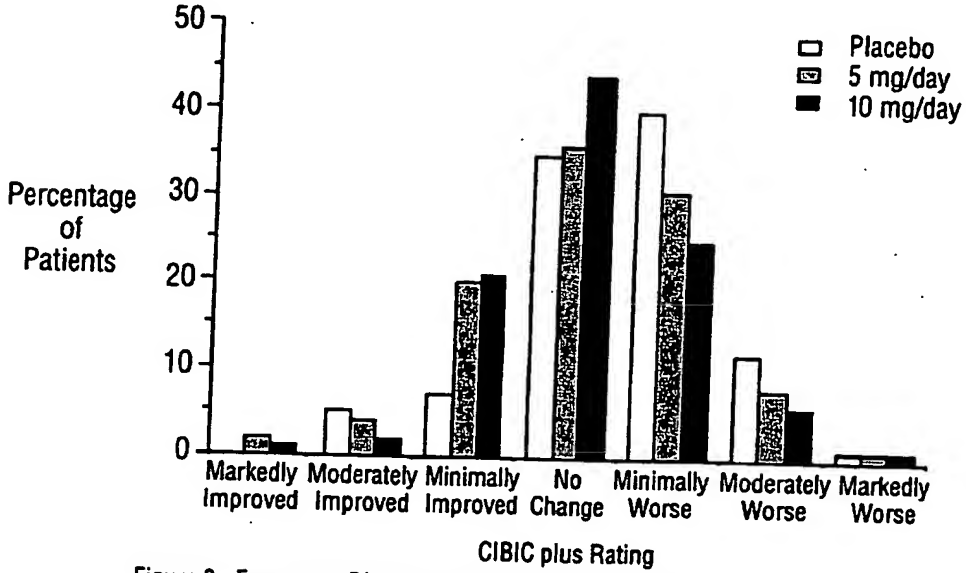


Figure 3. Frequency Distribution of CIBIC plus Scores at Week 24

Fifteen-Week Study

In a study of 15 weeks duration, patients were randomized to receive single daily doses of placebo or either 5 mg/day or 10 mg/day of ARICEPT® for 12 weeks, followed by a 3-week placebo washout period. As in the 30-week study, to avoid acute cholinergic effects, the 10 mg/day treatment followed an initial 7-day treatment with 5 mg/day doses.

Effects on the ADAS-Cog: Figure 4 illustrates the time course of the change from baseline in ADAS-cog scores for all three dose groups over the 15 weeks of the study. After 12 weeks of treatment, the differences in mean ADAS-cog change scores for the ARICEPT® treated patients compared to the patients on placebo were 2.7 and 3.0 units each, for the 5 and 10 mg/day ARICEPT®treatment groups respectively. These differences were statistically significant. The effect size for the 10 mg/day group may appear to be slightly larger than that for 5 mg/day. However, the differences between active treatments were not statistically significant.

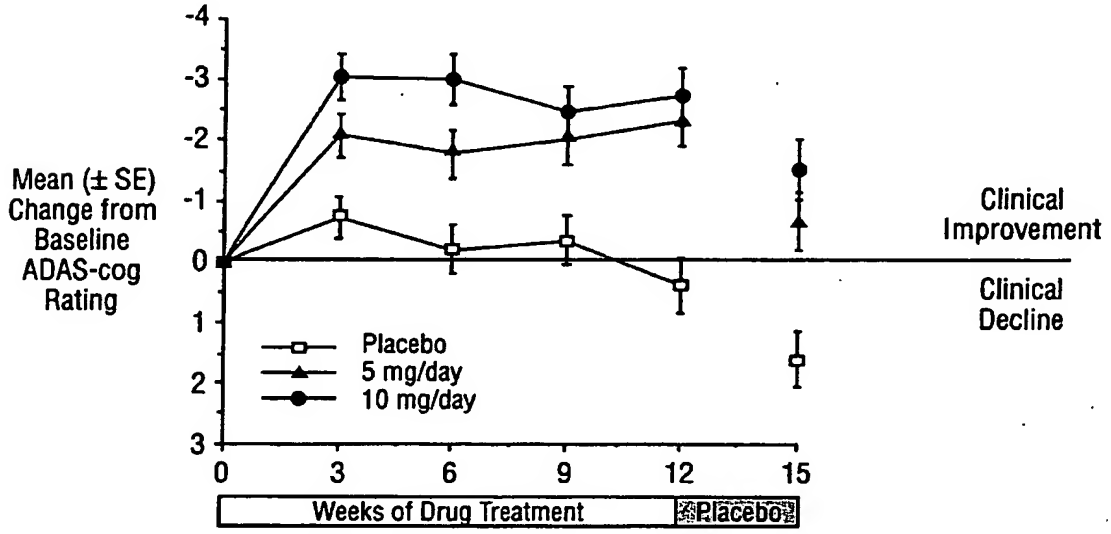


Figure 4. Time-course of the Change from Baseline in ADAS-cog Score for Patients Completing the 15-week Study.

Following 3 weeks of placebo washout, scores on the ADAS-cog for both the ARICEPT® treatment groups increased, indicating that discontinuation of ARICEPT® resulted in a loss of its treatment effect. The duration of this placebo washout period was not sufficient to characterize the rate of loss of the treatment effect, but, the 30-week study (see above) demonstrated that treatment effects associated with the use of ARICEPT® abate within 6 weeks of treatment discontinuation.

Figure 5 illustrates the cumulative percentages of patients from each of the three treatment groups who attained the measure of improvement in ADAS-cog score shown

on the X axis. The same three change scores, (7-point and 4-point reductions from baseline or no change in score) as selected for the 30-week study have been used for this illustration. The percentages of patients achieving those results are shown in the inset table.

As observed in the 30-week study, the curves demonstrate that patients assigned to either placebo or to ARICEPT® have a wide range of responses, but that the ARICEPT® treated patients are more likely to show the greater improvements in cognitive performance.

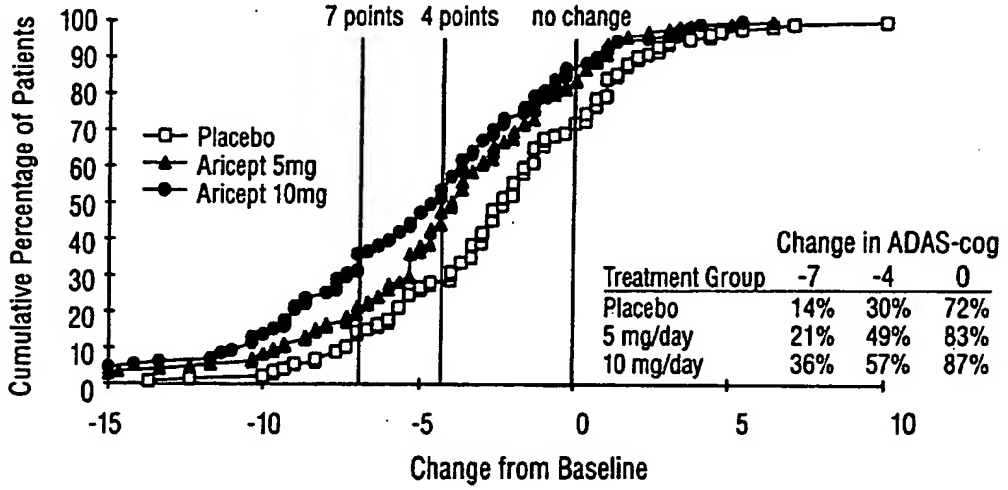


Figure 5. Cumulative Percentage of Patients with Specified Changes from Baseline ADAS-cog Scores. The Percentages of Randomized Patients Within Each Treatment Group Who Completed the Study Were: Placebo 93%, 5 mg/day 90% and 10 mg/day 82%.

Effects on the CIBIC plus: Figure 6 is a histogram of the frequency distribution of CIBIC plus scores attained by patients assigned to each of the three treatment groups who completed 12 weeks of treatment. The differences in mean scores for ARICEPT® treated patients compared to the patients on placebo at Week 12 were 0.36 and 0.38 units for the 5 mg/day and 10 mg/day treatment groups, respectively. These differences were statistically significant.

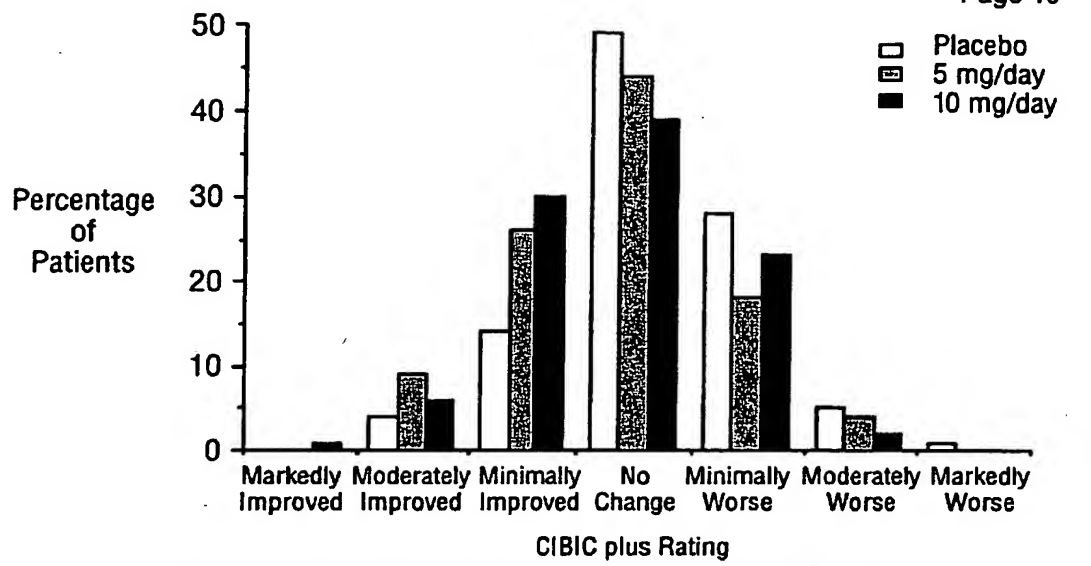


Figure 6. Frequency Distribution of CIBIC plus Scores at Week 12

In both studies, patient age, sex and race were not found to predict the clinical outcome of ARICEPT® treatment.

Clinical Pharmacokinetics

ARICEPT® Oral Solution is bioequivalent to ARICEPT® Tablets. Donepezil is well absorbed with a relative oral bioavailability of 100% and reaches peak plasma concentrations in 3 to 4 hours. Pharmacokinetics are linear over a dose range of 1-10 mg given once daily. Neither food nor time of administration (morning vs. evening dose) influences the rate or extent of absorption of ARICEPT® tablets. Administration of ARICEPT® Oral Solution to healthy volunteers with a high-fat meal decreased C_{max} by 17% and increased T_{max} by 1 hour, while the AUC_{0-72} was similar under fed and fasted conditions. This delay in absorption and decrease in exposure is not likely to be clinically significant; therefore, ARICEPT® Oral Solution can be taken without regard to meals.

The elimination half life of donepezil is about 70 hours and the mean apparent plasma clearance (Cl/F) is 0.13 L/hr/kg. Following multiple dose administration, donepezil accumulates in plasma by 4-7 fold and steady state is reached within 15 days. The steady state volume of distribution is 12 L/kg. Donepezil is approximately 96% bound to human plasma proteins, mainly to albumins (about 75%) and $alpha_1$ - acid glycoprotein (about 21%) over the concentration range of 2-1000 ng/mL.

Donepezil is both excreted in the urine intact and extensively metabolized to four major metabolites, two of which are known to be active, and a number of minor metabolites, not all of which have been identified. Donepezil is metabolized by CYP 450 isoenzymes 2D6 and 3A4 and undergoes glucuronidation. Following administration of ^{14}C-labeled

donepezil, plasma radioactivity, expressed as a percent of the administered dose, was present primarily as intact donepezil (53%) and as 6-O-desmethyl donepezil (11%), which has been reported to inhibit AChE to the same extent as donepezil *in vitro* and was found in plasma at concentrations equal to about 20% of donepezil. Approximately 57% and 15% of the total radioactivity was recovered in urine and feces, respectively, over a period of 10 days, while 28% remained unrecovered, with about 17% of the donepezil dose recovered in the urine as unchanged drug.

Special Populations:

Hepatic Disease: In a study of 10 patients with stable alcoholic cirrhosis, the clearance of ARICEPT® was decreased by 20% relative to 10 healthy age and sex matched subjects.

Renal Disease: In a study of 11 patients with moderate to severe renal impairment (Cl_{Cr} < 18 mL/min/1.73 m^2) the clearance of ARICEPT® did not differ from 11 age and sex matched healthy subjects.

Age: No formal pharmacokinetic study was conducted to examine age related differences in the pharmacokinetics of ARICEPT®. However, mean plasma ARICEPT® concentrations measured during therapeutic drug monitoring of elderly patients with Alzheimer's Disease are comparable to those observed in young healthy volunteers.

Gender and Race: No specific pharmacokinetic study was conducted to investigate the effects of gender and race on the disposition of ARICEPT®. However, retrospective pharmacokinetic analysis indicates that gender and race (Japanese and Caucasians) did not affect the clearance of ARICEPT®.

Drug-Drug Interactions

Drugs Highly Bound to Plasma Proteins: Drug displacement studies have been performed *in vitro* between this highly bound drug (96%) and other drugs such as furosemide, digoxin, and warfarin. ARICEPT® at concentrations of 0.3-10 μg/mL did not affect the binding of furosemide (5 μg/mL), digoxin (2 ng/mL), and warfarin (3 μg/mL) to human albumin. Similarly, the binding of ARICEPT® to human albumin was not affected by furosemide, digoxin and warfarin.

Effect of ARICEPT® on the Metabolism of Other Drugs: No *in vivo* clinical trials have investigated the effect of ARICEPT® on the clearance of drugs metabolized by CYP 3A4 (e.g. cisapride, terfenadine) or by CYP 2D6 (e.g. imipramine). However, *in vitro* studies show a low rate of binding to these enzymes (mean K_i about 50-130 μM), that, given the therapeutic plasma concentrations of donepezil (164 nM), indicates little likelihood of interference.

Whether ARICEPT® has any potential for enzyme induction is not known.

Formal pharmacokinetic studies evaluated the potential of ARICEPT® for interaction with theophylline, cimetidine, warfarin, digoxin and ketoconazole. No effects of ARICEPT® on the pharmacokinetics of these drugs were observed.

Effect of Other Drugs on the Metabolism of ARICEPT®: Ketoconazole and quinidine, inhibitors of CYP450, 3A4 and 2D6, respectively, inhibit donepezil metabolism *in vitro*. Whether there is a clinical effect of quinidine is not known. In a 7-day crossover study in 18 healthy volunteers, ketoconazole (200mg q.d.) increased mean donepezil (5mg q.d.) concentrations (AUC_{0-24} and C_{max}) by 36%. The clinical relevance of this increase in concentration is unknown.

Inducers of CYP 2D6 and CYP 3A4 (e.g., phenytoin, carbamazepine, dexamethasone, rifampin, and phenobarbital) could increase the rate of elimination of ARICEPT®.

Formal pharmacokinetic studies demonstrated that the metabolism of ARICEPT® is not significantly affected by concurrent administration of digoxin or cimetidine.

INDICATIONS AND USAGE

ARICEPT® is indicated for the treatment of mild to moderate dementia of the Alzheimer's type.

CONTRAINDICATIONS

ARICEPT® is contraindicated in patients with known hypersensitivity to donepezil hydrochloride or to piperidine derivatives.

WARNINGS

Anesthesia: ARICEPT®, as a cholinesterase inhibitor, is likely to exaggerate succinylcholine-type muscle relaxation during anesthesia.

Cardiovascular Conditions: Because of their pharmacological action, cholinesterase inhibitors may have vagotonic effects on the sinoatrial and atrioventricular nodes. This effect may manifest as bradycardia or heart block in patients both with and without known underlying cardiac conduction abnormalities. Syncopal episodes have been reported in association with the use of ARICEPT®.

Gastrointestinal Conditions: Through their primary action, cholinesterase inhibitors may be expected to increase gastric acid secretion due to increased cholinergic activity. Therefore, patients should be monitored closely for symptoms of active or occult gastrointestinal bleeding, especially those at increased risk for developing ulcers, e.g., those with a history of ulcer disease or those receiving concurrent nonsteroidal anti-inflammatory drugs (NSAIDS). Clinical studies of ARICEPT® have shown no increase,

relative to placebo, in the incidence of either peptic ulcer disease or gastrointestinal bleeding.

ARICEPT®, as a predictable consequence of its pharmacological properties, has been shown to produce diarrhea, nausea and vomiting. These effects, when they occur, appear more frequently with the 10 mg/day dose than with the 5 mg/day dose. In most cases, these effects have been mild and transient, sometimes lasting one to three weeks, and have resolved during continued use of ARICEPT®.

Genitourinary: Although not observed in clinical trials of ARICEPT®, cholinomimetics may cause bladder outflow obstruction.

Neurological Conditions: Seizures: Cholinomimetics are believed to have some potential to cause generalized convulsions. However, seizure activity also may be a manifestation of Alzheimer's Disease.

Pulmonary Conditions: Because of their cholinomimetic actions, cholinesterase inhibitors should be prescribed with care to patients with a history of asthma or obstructive pulmonary disease.

Sulfites:

ARICEPT® Oral Solution contains sodium metabisulfite, a sulfite that may cause allergic-type reactions including anaphylactic symptoms and life-threatening or less severe asthmatic episodes in certain susceptible people. The overall prevalence of sulfite sensitivity in the general population is unknown and probably low. Sulfite sensitivity is seen more frequently in asthmatic than nonasthmatic people.

PRECAUTIONS

Drug-Drug Interactions (see Clinical Pharmacology: Clinical Pharmacokinetics: Drug-drug Interactions)

Effect of ARICEPT® on the Metabolism of Other Drugs: No *in vivo* clinical trials have investigated the effect of ARICEPT® on the clearance of drugs metabolized by CYP 3A4 (e.g. cisapride, terfenadine) or by CYP 2D6 (e.g. imipramine). However, *in vitro* studies show a low rate of binding to these enzymes (mean K_i about 50-130 μM), that, given the therapeutic plasma concentrations of donepezil (164 nM), indicates little likelihood of interference.

Whether ARICEPT® has any potential for enzyme induction is not known.

Formal pharmacokinetic studies evaluated the potential of ARICEPT® for interaction with theophylline, cimetidine, warfarin, digoxin and ketoconazole. No effects of ARICEPT® on the pharmacokinetics of these drugs were observed.

Effect of Other Drugs on the Metabolism of ARICEPT®: Ketoconazole and quinidine, inhibitors of CYP450, 3A4 and 2D6, respectively, inhibit donepezil metabolism *in vitro*. Whether there is a clinical effect of quinidine is not known. In a 7-day crossover study in 18 healthy volunteers, ketoconazole (200mg q.d.) increased mean donepezil (5mg q.d.) concentrations (AUC_{0-24} and C_{max}) by 36%. The clinical relevance of this increase in concentration is unknown.

Inducers of CYP 2D6 and CYP 3A4 (e.g., phenytoin, carbamazepine, dexamethasone, rifampin, and phenobarbital) could increase the rate of elimination of ARICEPT®.

Formal pharmacokinetic studies demonstrated that the metabolism of ARICEPT® is not significantly affected by concurrent administration of digoxin or cimetidine.

Use with Anticholinergics: Because of their mechanism of action, cholinesterase inhibitors have the potential to interfere with the activity of anticholinergic medications.

Use with Cholinomimetics and Other Cholinesterase Inhibitors: A synergistic effect may be expected when cholinesterase inhibitors are given concurrently with succinylcholine, similar neuromuscular blocking agents or cholinergic agonists such as bethanechol.

Carcinogenesis, Mutagenesis, Impairment of Fertility

No evidence of a carcinogenic potential was obtained in an 88-week carcinogenicity study of donepezil hydrochloride conducted in CD-1 mice at doses up to 180 mg/kg/day (approximately 90 times the maximum recommended human dose on a mg/m^2 basis), or in a 104-week carcinogenicity study in Sprague-Dawley rats at doses up to 30mg/kg/day (approximately 30 times the maximum recommended human dose on a mg/m^2 basis).

Donepezil was not mutagenic in the Ames reverse mutation assay in bacteria, or in a mouse lymphoma forward mutation assay *in vitro*. In the chromosome aberration test in cultures of Chinese hamster lung (CHL) cells, some clastogenic effects were observed. Donepezil was not clastogenic in the *in vivo* mouse micronucleus test and was not genotoxic in an *in vivo* unscheduled DNA synthesis assay in rats.

Donepezil had no effect on fertility in rats at doses up to 10 mg/kg/day (approximately 8 times the maximum recommended human dose on a mg/m^2 basis).

Pregnancy

Pregnancy Category C: Teratology studies conducted in pregnant rats at doses up to 16 mg/kg/day (approximately 13 times the maximum recommended human dose on a mg/m^2 basis) and in pregnant rabbits at doses up to 10 mg/kg/day (approximately 16 times the maximum recommended human dose on a mg/m^2 basis) did not disclose any evidence for a teratogenic potential of donepezil. However, in a study in which pregnant

rats were given up to 10 mg/kg/day (approximately 8 times the maximum recommended human dose on a mg/m^2 basis) from day 17 of gestation through day 20 postpartum, there was a slight increase in still births and a slight decrease in pup survival through day 4 postpartum at this dose; the next lower dose tested was 3 mg/kg/day. There are no adequate or well-controlled studies in pregnant women. ARICEPT® should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nursing Mothers

It is not known whether donepezil is excreted in human breast milk. ARICEPT® has no indication for use in nursing mothers.

Pediatric Use

There are no adequate and well-controlled trials to document the safety and efficacy of ARICEPT® in any illness occurring in children.

Geriatric Use

Alzheimer's disease is a disorder occurring primarily in individuals over 55 years of age. The mean age of patients enrolled in the clinical studies with ARICEPT® was 73 years; 80% of these patients were between 65 and 84 years old and 49% of patients were at or above the age of 75. The efficacy and safety data presented in the clinical trials section were obtained from these patients. There were no clinically significant differences in most adverse events reported by patient groups ≥ 65 years old and < 65 years old.

ADVERSE REACTIONS

Adverse Events Leading to Discontinuation

The rates of discontinuation from controlled clinical trials of ARICEPT® due to adverse events for the ARICEPT® 5 mg/day treatment groups were comparable to those of placebo-treatment groups at approximately 5%. The rate of discontinuation of patients who received 7-day escalations from 5 mg/day to 10 mg/day, was higher at 13%.

The most common adverse events leading to discontinuation, defined as those occurring in at least 2% of patients and at twice the incidence seen in placebo patients, are shown in Table 1.

Table 1. Most Frequent Adverse Events Leading to Withdrawal from Controlled Clinical Trials by Dose Group

Dose Group	Placebo	5 mg/day ARICEPT®	10 mg/day ARICEPT®
Patients Randomized	355	350	315
Event/%Discontinuing			
Nausea	1%	1%	3%
Diarrhea	0%	<1%	3%
Vomiting	<1%	<1%	2%

Most Frequent Adverse Clinical Events Seen in Association with the Use of ARICEPT®

The most common adverse events, defined as those occurring at a frequency of at least 5% in patients receiving 10 mg/day and twice the placebo rate, are largely predicted by ARICEPT®'s cholinomimetic effects. These include nausea, diarrhea, insomnia, vomiting, muscle cramp, fatigue and anorexia. These adverse events were often of mild intensity and transient, resolving during continued ARICEPT® treatment without the need for dose modification.

There is evidence to suggest that the frequency of these common adverse events may be affected by the rate of titration. An open-label study was conducted with 269 patients who received placebo in the 15 and 30-week studies. These patients were titrated to a dose of 10 mg/day over a 6-week period. The rates of common adverse events were lower than those seen in patients titrated to 10 mg/day over one week in the controlled clinical trials and were comparable to those seen in patients on 5 mg/day.

See Table 2 for a comparison of the most common adverse events following one and six week titration regimens.

Table 2. Comparison of rates of adverse events in patients titrated to 10 mg/day over 1 and 6 weeks

	No titration		One week titration	Six week titration
Adverse Event	**Placebo (n=315)**	**5 mg/day (n=311)**	**10 mg/day (n=315)**	**10 mg/day (n=269)**
Nausea	6%	5%	19%	6%
Diarrhea	5%	8%	15%	9%
Insomnia	6%	6%	14%	6%
Fatigue	3%	4%	8%	3%
Vomiting	3%	3%	8%	5%
Muscle cramps	2%	6%	8%	3%
Anorexia	2%	3%	7%	3%

Adverse Events Reported in Controlled Trials

The events cited reflect experience gained under closely monitored conditions of clinical trials in a highly selected patient population. In actual clinical practice or in other clinical trials, these frequency estimates may not apply, as the conditions of use, reporting behavior, and the kinds of patients treated may differ. Table 3 lists treatment emergent signs and symptoms that were reported in at least 2% of patients in placebo-controlled trials who received ARICEPT® and for which the rate of occurrence was greater for ARICEPT® assigned than placebo assigned patients. In general, adverse events occurred more frequently in female patients and with advancing age.

Table 3. Adverse Events Reported in Controlled Clinical Trials in at Least 2% of Patients Receiving ARICEPT® and at a Higher Frequency than Placebo-treated Patients		
Body System/Adverse Event	**Placebo (n=355)**	**ARICEPT® (n=747)**
Percent of Patients with any Adverse Event	**72**	**74**
Body as a Whole		
Headache	9	10
Pain, various locations	8	9
Accident	6	7
Fatigue	3	5
Cardiovascular System		
Syncope	1	2
Digestive System		
Nausea	6	11
Diarrhea	5	10
Vomiting	3	5
Anorexia	2	4
Hemic and Lymphatic System		
Ecchymosis	3	4
Metabolic and Nutritional Systems		
Weight Decrease	1	3
Musculoskeletal System		
Muscle Cramps	2	6
Arthritis	1	2
Nervous System		
Insomnia	6	9
Dizziness	6	8
Depression	<1	3
Abnormal Dreams	0	3
Somnolence	<1	2
Urogenital System		
Frequent Urination	1	2

Other Adverse Events Observed During Clinical Trials

ARICEPT® has been administered to over 1700 individuals during clinical trials worldwide. Approximately 1200 of these patients have been treated for at least 3 months and more than 1000 patients have been treated for at least 6 months. Controlled and uncontrolled trials in the United States included approximately 900 patients. In regards to the highest dose of 10 mg/day, this population includes 650 patients treated for 3 months, 475 patients treated for 6 months and 116 patients treated for over 1 year. The range of patient exposure is from 1 to 1214 days.

Treatment emergent signs and symptoms that occurred during 3 controlled clinical trials and two open-label trials in the United States were recorded as adverse events by the clinical investigators using terminology of their own choosing. To provide an overall estimate of the proportion of individuals having similar types of events, the events were

grouped into a smaller number of standardized categories using a modified COSTART dictionary and event frequencies were calculated across all studies. These categories are used in the listing below. The frequencies represent the proportion of 900 patients from these trials who experienced that event while receiving ARICEPT®. All adverse events occurring at least twice are included, except for those already listed in Tables 2 or 3, COSTART terms too general to be informative, or events less likely to be drug caused. Events are classified by body system and listed using the following definitions: *frequent adverse events* - those occurring in at least 1/100 patients; *infrequent adverse events* - those occurring in 1/100 to 1/1000 patients. These adverse events are not necessarily related to ARICEPT® treatment and in most cases were observed at a similar frequency in placebo-treated patients in the controlled studies. No important additional adverse events were seen in studies conducted outside the United States.

Body as a Whole: *Frequent:* influenza, chest pain, toothache; *Infrequent:* fever, edema face, periorbital edema, hernia hiatal, abscess, cellulitis, chills, generalized coldness, head fullness, listlessness.

Cardiovascular System: *Frequent:* hypertension, vasodilation, atrial fibrillation, hot flashes, hypotension; *Infrequent:* angina pectoris, postural hypotension, myocardial infarction, AV block (first degree), congestive heart failure, arteritis, bradycardia, peripheral vascular disease, supraventricular tachycardia, deep vein thrombosis.

Digestive System: *Frequent:* fecal incontinence, gastrointestinal bleeding, bloating, epigastric pain; *Infrequent:* eructation, gingivitis, increased appetite, flatulence, periodontal abscess, cholelithiasis, diverticulitis, drooling, dry mouth, fever sore, gastritis, irritable colon, tongue edema, epigastric distress, gastroenteritis, increased transaminases, hemorrhoids, ileus, increased thirst, jaundice, melena, polydipsia, duodenal ulcer, stomach ulcer.

Endocrine System: *Infrequent:* diabetes mellitus, goiter.

Hemic and Lymphatic System: *Infrequent:* anemia, thrombocythemia, thrombocytopenia, eosinophilia, erythrocytopenia.

Metabolic and Nutritional Disorders: *Frequent:* dehydration; *Infrequent:* gout, hypokalemia, increased creatine kinase, hyperglycemia, weight increase, increased lactate dehydrogenase.

Musculoskeletal System: *Frequent:* bone fracture; *Infrequent:* muscle weakness, muscle fasciculation.

Nervous System: *Frequent:* delusions, tremor, irritability, paresthesia, aggression, vertigo, ataxia, increased libido, restlessness, abnormal crying, nervousness, aphasia; *Infrequent:* cerebrovascular accident, intracranial hemorrhage, transient ischemic attack, emotional lability, neuralgia, coldness (localized), muscle spasm, dysphoria, gait abnormality, hypertonia, hypokinesia, neurodermatitis, numbness (localized), paranoia,

dysarthria, dysphasia, hostility, decreased libido, melancholia, emotional withdrawal, nystagmus, pacing.

Respiratory System: *Frequent:* dyspnea, sore throat, bronchitis; *Infrequent:* epistaxis, post nasal drip, pneumonia, hyperventilation, pulmonary congestion, wheezing, hypoxia, pharyngitis, pleurisy, pulmonary collapse, sleep apnea, snoring.

Skin and Appendages: *Frequent:* pruritus, diaphoresis, urticaria; *Infrequent:* dermatitis, erythema, skin discoloration, hyperkeratosis, alopecia, fungal dermatitis, herpes zoster, hirsutism, skin striae, night sweats, skin ulcer.

Special Senses: *Frequent:* cataract, eye irritation, vision blurred; *Infrequent:* dry eyes, glaucoma, earache, tinnitus, blepharitis, decreased hearing, retinal hemorrhage, otitis externa, otitis media, bad taste, conjunctival hemorrhage, ear buzzing, motion sickness, spots before eyes.

Urogenital System: *Frequent:* urinary incontinence, nocturia; *Infrequent:* dysuria, hematuria, urinary urgency, metrorrhagia, cystitis, enuresis, prostate hypertrophy, pyelonephritis, inability to empty bladder, breast fibroadenosis, fibrocystic breast, mastitis, pyuria, renal failure, vaginitis.

Postintroduction Reports

Voluntary reports of adverse events temporally associated with ARICEPT® that have been received since market introduction that are not listed above, and that there is inadequate data to determine the causal relationship with the drug include the following: abdominal pain, agitation, cholecystitis, confusion, convulsions, hallucinations, heart block (all types), hemolytic anemia, hepatitis, hyponatremia, neuroleptic malignant syndrome, pancreatitis, and rash.

OVERDOSAGE

Because strategies for the management of overdose are continually evolving, it is advisable to contact a Poison Control Center to determine the latest recommendations for the management of an overdose of any drug.

As in any case of overdose, general supportive measures should be utilized. Overdosage with cholinesterase inhibitors can result in cholinergic crisis characterized by severe nausea, vomiting, salivation, sweating, bradycardia, hypotension, respiratory depression, collapse and convulsions. Increasing muscle weakness is a possibility and may result in death if respiratory muscles are involved. Tertiary anticholinergics such as atropine may be used as an antidote for ARICEPT® overdosage. Intravenous atropine sulfate titrated to effect is recommended: an initial dose of 1.0 to 2.0 mg IV with subsequent doses based upon clinical response. Atypical responses in blood pressure and heart rate have been reported with other cholinomimetics when co-administered with quaternary anticholinergics such as glycopyrrolate. It is not known whether

ARICEPT® and/or its metabolites can be removed by dialysis (hemodialysis, peritoneal dialysis, or hemofiltration).

Dose-related signs of toxicity in animals included reduced spontaneous movement, prone position, staggering gait, lacrimation, clonic convulsions, depressed respiration, salivation, miosis, tremors, fasciculation and lower body surface temperature.

DOSAGE AND ADMINISTRATION

The dosages of ARICEPT® shown to be effective in controlled clinical trials are 5 mg and 10 mg administered once per day.

The higher dose of 10 mg did not provide a statistically significantly greater clinical benefit than 5 mg. There is a suggestion, however, based upon order of group mean scores and dose trend analyses of data from these clinical trials, that a daily dose of 10 mg of ARICEPT® might provide additional benefit for some patients. Accordingly, whether or not to employ a dose of 10 mg is a matter of prescriber and patient preference.

Evidence from the controlled trials indicates that the 10 mg dose, with a one week titration, is likely to be associated with a higher incidence of cholinergic adverse events than the 5 mg dose. In open label trials using a 6 week titration, the frequency of these same adverse events was similar between the 5 mg and 10 mg dose groups. Therefore, because steady state is not achieved for 15 days and because the incidence of untoward effects may be influenced by the rate of dose escalation, treatment with a dose of 10 mg should not be contemplated until patients have been on a daily dose of 5 mg for 4 to 6 weeks.

Each teaspoon (5 mL) of ARICEPT® Oral Solution contains a 5 mg dose. Patients should be instructed as to how to measure their dose of ARICEPT® Oral Solution in teaspoons.

ARICEPT® Oral Solution should be taken in the evening, just prior to retiring. ARICEPT® Oral Solution can be taken with or without food.

HOW SUPPLIED

ARICEPT® Oral Solution is a clear, colorless to light yellow solution containing 1 mg of donepezil hydrochloride in each mL (1 mg/mL). Each teaspoon (5mL) contains 5 mg of donepezil hydrochloride.

NDC#62856-851-30 300 mL HDPE Bottles

R_x only

ARICEPT® is a registered trademark of
Eisai Co., Ltd.
Manufactured and Marketed by Eisai Inc., Teaneck, NJ 07666
Marketed by
Pfizer Inc, New York, NY 10017

Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 12016–12021, September 1998
Neurobiology

Altered thalamic response to levodopa in Parkinson's patients with dopa-induced dyskinesias

TAMARA HERSHEY*, KEVIN J. BLACK*†‡, MIKULA K. STAMBUK§, JUANITA L. CARL‡, LORI A. MCGEE-MINNICH‡, AND JOEL S. PERLMUTTER†‡¶

Departments of *Psychiatry, †Radiology, ‡Neurology and Neurological Surgery, §Washington University School of Medicine, St. Louis, MO 63110

Communicated by Marcus E. Raichle, Washington University School of Medicine, St. Louis, MO, July 28, 1998 (received for review February 3, 1998)

ABSTRACT **Parkinson's disease (PD) is a progressive neurologic condition characterized by tremor, slowness, stiffness, and unstable posture. Degeneration of dopamine-producing neurons in the substantia nigra causes PD. Treatment with levodopa, a precursor of dopamine, initially ameliorates the clinical manifestations of PD. However, chronic levodopa treatment can produce severe involuntary movements (so-called dopa-induced dyskinesias or DID), limiting treatment. Pallidotomy, placement of a surgical lesion in the internal segment of the globus pallidus, reduces DID. Because this result is inconsistent with current theories of both basal ganglia function and DID, it prompted us to investigate the brain's response to levodopa. We measured regional cerebral blood flow response to levodopa with positron-emission tomography in 6 PD patients with DID, 10 chronically treated PD patients without DID, 17 dopa-naïve PD patients, and 11 normals. The dose of levodopa was chosen to produce clinical benefit without inducing DID. This strategy allowed us to examine the brain response to levodopa across groups without the confounding effect of differences in motor behavior. We found that the DID group had a significantly greater response in ventrolateral thalamus than the other groups. This was associated with decreased activity in primary motor cortex. These findings are consistent with increased inhibitory output from the internal segment of the globus pallidus to thalamus after levodopa administration. They provide a physiological explanation for the clinical efficacy of pallidotomy and new insights into the physiology of the basal ganglia.**

Parkinson's disease (PD), a disease of brain dopamine deficiency, results from the degeneration of nigrostriatal neurons (1). Levodopa, the immediate precursor of dopamine, is the primary treatment for PD. Initially, treatment with levodopa ameliorates the clinical manifestations of PD such as slowness or reduction of spontaneous movement (bradykinesia or akinesia), tremor, rigidity, and an unstable posture (2). Many patients, however, develop a debilitating side effect of chronic levodopa treatment, consisting of drug-induced involuntary movements (dopa-induced dyskinesias, or DID). The development of DID severely limits effective treatment with levodopa.

Recent studies have shown that pallidotomy surgery, the placement of an electrolytic lesion in the internal segment of the globus pallidus (GPi), consistently decreases DID (3). Thus, the presence and severity of DID have become important selection criteria for patients undergoing pallidotomy (3). However, it is puzzling that pallidotomy's favorable effect on DID is inconsistent with current theories of basal ganglia function used to explain the mechanism of DID (4).

The output of the basal ganglia is through the GPi. GPi, in turn, sends projection neurons to thalamus thought to tonically inhibit thalamocortical circuits, including those involved in motor activity. Currently, some investigators believe that levodopa causes underactivity of GPi neurons thereby releasing the inhibition of thalamocortical motor circuits, which leads to the production of involuntary movement in patients with DID (5). According to the commonly accepted model of basal ganglia function, pallidotomy would reduce the inhibitory output of the GPi even further, potentially increasing, rather than decreasing DID. This contradiction challenges our understanding of basal ganglia physiology and the pathophysiology of DID (4). Therefore, determining the neurophysiological abnormalities underlying DID may provide new understanding of basal ganglia function, as well as enhance our understanding of pallidotomy surgery.

The present study addresses the question of how blood flow responses to an acute dose of levodopa differ between PD patients who have developed DID and those who have not. Changes in regional cerebral blood flow are thought to reflect neuronal activity, primarily in axonal terminal fields (6). Positron emission tomography (PET) permits the *in vivo* assessment of regional cerebral blood flow responses to pharmacologic challenges (7–15) and thus is a useful method for investigating DID. Because DID occur in response to levodopa, we chose to assess neurophysiological responses to an acute dose of levodopa.

MATERIALS AND METHODS

Subjects. *PD patients.* Patients with clinically diagnosed idiopathic PD (16) were recruited primarily from the Movement Disorders Center at Washington University School of Medicine. Patients were excluded for any evidence of secondary parkinsonism (e.g., drug-induced or atypical presentation), dementia (Mini-Mental Status Exam score <26) (17), depression (Hamilton Depression Scale score > 10) (18), history of other neurological disorders, psychiatric disorders, substance abuse, neuroleptic use, or suspicion of pregnancy. All of the patients were right-handed (19) and 21 were male and 12 were female. Three groups of patients were specifically recruited: patients with no history of levodopa or other dopamine agonist treatment (dopa-naïve, $n = 17$), patients on chronic levodopa treatment but without DID ($n = 10$) and patients on chronic levodopa treatment with DID ($n = 7$). All of the patients had typical features of clinically defined PD, including patients who had not developed DID. Approximately 70% of patients remain DID-free after several years of treatment (20).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: PD, Parkinson's disease; DID, dopa-induced dyskinesias; PET, positron-emission tomography; GPi, internal segment of the globus pallidus; SNpr, substantia nigra, pars reticulata; STN, subthalamic nucleus; UPDRS, Unified Parkinson's Disease Rating Scale.

¶To whom reprint requests should be addressed. e-mail: joel@npg.wustl.edu.

One DID patient was excluded from analysis because he had dyskinesias during his post-levodopa scans. This strategy allowed us to look at the physiological response to levodopa *without* the confounding effect of differences in motor behavior.

Twenty-one (64%) of our 33 PD patients had greater motor symptoms on the right side of the body, whereas only 12 (36%) had greater motor symptoms on the left side of the body. In addition, within the DID group, five of six patients had greater motor and DID symptoms on the right side of the body. Eight dopa-naïve PD patients had unilateral motor symptoms; the remaining patients had bilateral motor symptoms. See Table 1 for additional information.

Normals. Eleven right-handed women and three right-handed men without PD were recruited by using the same exclusionary criteria as outlined above. One woman and two men were excluded from analysis because their plasma levodopa levels were extremely low (average post-levodopa plasma levels <400 ng/ml), suggesting poor enteral absorption of the drug. See Table 1 for additional information.

Written informed consent was obtained from all subjects before their participation in the study. The study protocol was approved by the Radioactive Drug Research Committee and the Human Studies Committee of Washington University School of Medicine.

Protocol. All PD patients on levodopa therapy refrained from taking levodopa for at least 12 hr before participation in the PET protocol. At the beginning of the study, all subjects took carbidopa 200 mg orally and had a baseline clinical evaluation consisting of the Unified Parkinson's Disease rating scale (UPDRS) (21) and the modified Hoehn & Yahr rating scale (16). They then were placed in the scanner, a 20-gauge catheter was inserted into an arm vein to permit injection of $H_2{}^{15}O$, and in some subjects a similar catheter was inserted into the radial artery at the wrist after local lidocaine anesthesia for arterial blood sampling. An individually molded polyform mask helped stabilize each subject's head within the scanner. Radio-opaque markers placed in the external auditory canals and a lateral skull radiograph with the center PET slice indicated by a radio-opaque wire provided a record of the subjects' exact head position in relation to the PET (22).

Next, baseline blood samples were taken, and two to three baseline 40-sec PET measurements of blood flow (23) were obtained 15 min apart, as described below. Levodopa/carbidopa then was given orally (150 mg/37.5 mg). We chose a dose of levodopa that was generally lower than the DID patients' usual doses, and thus was unlikely to induce dyskinesias. Patients were observed for movements by at least one movement disorders specialist during each scan. One DID patient was excluded from analysis due to dyskinesias during the scans. This strategy allowed us to look at the physiological response to levodopa across groups without the confounding additional effect of a difference in motor behavior during the scans between groups. Approximately 45–75 min after levodopa, two to three additional blood flow measurements were obtained. Clinical ratings (modified motor UPDRS including ratings for tremor, rigidity, bradykinesia, and tapping speed for upper extremities; 16 total possible points) and blood samples were obtained at the time of each scan and every 15 min after administration of levodopa. During each PET scan, the room was darkened and subjects' eyes were closed.

PET Methods. All PET studies were performed on a Siemens 953B scanner (CTI, Knoxville, TN) in 2-dimensional mode. Data were recorded simultaneously for 31 slices with a center-to-center slice separation of 3.4 mm (24). After subjects were positioned, a transmission scan used for individual attenuation correction was acquired with rotating rod sources containing $^{68}Ge/^{68}Ga$. PET images were reconstructed with a transverse resolution of 14 mm full width half maximum. Blood flow was measured by using a 40-sec emission scan after the i.v. bolus injection of 5–10 ml of saline containing 40–50 mCi (1 Ci = 37 GBq) of ^{15}O-labeled water (23, 25, 26).

Levodopa Measurements. Levodopa and carbidopa levels were measured by using HPLC with electrochemical detection following a modified version of published methods (27, 28). We added an internal standard, 3,4-dihydrobenzylamine (DHBA), to simplify quantification. Although our primary interest was in levodopa, in the course of developing the method we found that it was possible to simultaneously measure carbidopa as well.

PET Data Analysis. To minimize artifact from movement between scans, PET images for each subject first were aligned to a baseline scan from that subject's scan series by using an automated method (29). All scans then were placed into Talairach atlas space (30). We analyzed all regional data by using normalized PET counts, which are linearly related to quantitative regional cerebral blood flow (31).

The data analysis strategy was designed to determine which regional brain responses to levodopa differentiate DID from non-DID patients. This strategy also was designed to minimize Type 1, or false positive errors, to ensure that each finding is reliable (32, 33). Thus, our strategy may not detect other lower-level responses.

We first determined which regions were reliably affected by levodopa activation in DID and non-DID patients. For each group, we randomly selected one pre-levodopa scan and one post-levodopa scan from each subject in that group and subtracted the pre- from the post-levodopa scan. We then created a composite subtraction image for each group averaging the subtraction pairs from each group. The composite subtraction images from the two groups were used to generate hypotheses about which regions of the brain responded to levodopa.

We used an automatic search routine (34) to identify peak responses in these two composite hypothesis-generating subtraction images. Candidate regions were selected from this

Table 1. Demographic and clinical variables: mean (SD)

	Parkinson's disease groups			
	Normals	Dopa-naive	Dopa-treated with no dyskinesias	Dopa-treated with DID
n	11	17	10	6
Age	53.6 (14.4)	60.9 (14.2)	69.9 (3.2)*	60.3 (8.0)
Hamilton depression scale	1.5 (1.6)	2.6 (2.5)	1.1 (2.8)	2.5 (3.1)
Mini-mental status score	29.6 (0.7)	28.8 (1.9)	28.7 (1.3)	28.7 (2.3)
Hoehn and Yahr stage		1.7 (0.3)	2.6 (0.6)†	2.4 (0.8)†
Symptom duration, years		2.8 (2.9)	7.6 (6.4)	10.8 (6.5)†
Modified UPDRS change		−2.7 (2.2)	−3.6 (3.3)	−3.0 (3.4)
Treatment duration, years			5.9 (5.7)	7.8 (6.8)

*Significantly different from normals ($P < 0.05$).
†Significantly different from dopa-naive group ($P < 0.05$).

peak-search if they (*i*) had >8% change in magnitude and (*ii*) had relevance to dopaminergic pathways. For the non-DID group, we selected three regions and for the DID group, we selected five regions. Once these candidate regions were selected for each group, the paired scans used to create these images were discarded. We then tested the statistical significance of the candidate responses by using the remaining scans in each group. To determine this, a 10-mm diameter sphere-shaped volume of interest was centered on the coordinates of the candidate regions in the remaining pre- and post-levodopa scans. This size was chosen to best approximate the search volume used in the peak identification routine and to reduce partial volume contributions from nearby regions.

The candidate regions selected from the hypothesis-generating image from non-DID patients were examined in the remaining scans from these patients and the normal subjects. Mean changes in blood flow were calculated for each of these candidate regions in the remaining scans. One-tailed *t* tests were conducted on the mean changes in blood flow. Critical *P* values were Bonferroni-corrected to minimize type I error. If a candidate region reached significance, it was accepted as a reliably activated region for the group.

The candidate regions selected from the hypothesis-generating image from DID patients were examined in the remaining scans from the DID patients and tested for statistical significance precisely as done for the non-DID patients.

To determine how DID and non-DID patients differed in their response to levodopa, we took the reliably activated regions from the above analysis and examined them for differences across groups. These regions were applied to all groups, using all scans to generate mean pre- and post-levodopa values for each subject.

The mean blood flow values for each region were analyzed by using repeated measures general linear models with group as the between-subjects variable and drug (pre- vs. post-levodopa) as the repeated variable. These analyses were performed to determine whether there were any significant interactions between group and drug, indicating that groups responded to levodopa differently. We particularly were interested in whether the DID patients responded to levodopa differently compared with non-DID patients in any region. Appropriate post-hoc comparisons were used to examine any significant main effects or interactions in more detail. In addition, correlations were performed between relevant clinical variables, plasma levodopa levels, and blood flow responses.

RESULTS

Peak Identification. In the non-DID group's composite subtraction image, only three regions of increased blood flow were found: The left putamen (−23, −5, 6), midbrain (stereotactic coordinates: −1 mm, −31 mm, −4 mm), and right amygdala (13, −5, −16). No regions with decreased blood flow were found. The statistical significance of these three candidate regions of increased blood flow was confirmed in the remaining scans for the non-DID patients (*t* tests, *P*s < 0.01; see Table 2).

Table 2. Levodopa-induced mean percent change (SD) in blood flow in hypothesis testing data sets

	% Change	*t* Value	*P* value
Left putamen	4.6 (10.3)	3.33	0.001*
Midbrain	8.7 (13.1)	4.88	<0.001*
Right amygdala	4.6 (13.2)	2.56	<0.01*
Left thalamus	18.9 (18.2)	2.76	0.017†
Left hippocampus	10.5 (9.8)	3.03	<0.01†

*Regions generated and tested in subjects without DID.
†Regions generated and tested in PD patients with DID.

From the DID group's composite subtraction image, we chose four candidate regions of increased blood flow in the left ventrolateral thalamus (stereotactic coordinates: −13 mm, −15 mm, 2 mm), left putamen (−25, 1, 10), right globus pallidus (25, −17, 2), and left hippocampus (−27, −25, −8). The left thalamic response had the highest magnitude change (13.9%) of all regions identified by the peak identification program. We chose only one candidate region, the right thalamus (13, −23, 8), with reduced blood flow. Of these five candidate regions (four increases and one decrease), the left thalamus (see Fig. 1) replicated at a borderline level (*t* test, $P = 0.017$; critical $P = 0.01$) and the left hippocampus significantly replicated (*t* test, $P < .01$).

Group Comparisons. To determine differences among groups, all regions found to be reliably activated in the hypothesis-testing step for either group were examined. These regions were the three regions from the non-DID group (left putamen, midbrain, and right amygdala) and the two regions from the DID group (left thalamus and left hippocampus). Each region was analyzed with a repeated measures general linear model, with drug (pre- vs. post-levodopa flow) as the repeated measure and group (normals, dopa-naïve, dopa-treated, and DID) as the between-subjects variable. Results from these five repeated measures analyses revealed the following:

1. Most importantly, there was a significant interaction between group and drug for the left thalamus region only [$F(3, 39)=4.17$, $P = 0.01$]. Post-hoc comparisons revealed that DID patients had a significantly greater increase in blood flow in this region compared with all other groups (see Fig. 2). Thus, the magnitude of the blood flow response to levodopa in the left thalamus statistically distinguished DID patients from all other groups. We explored the asymmetry of the thalamic response by comparing the left thalamic response with the response in the right thalamus (mirror image of the left thalamic volume of interest) by using paired *t* tests for each group of subjects. A significant difference was found for the

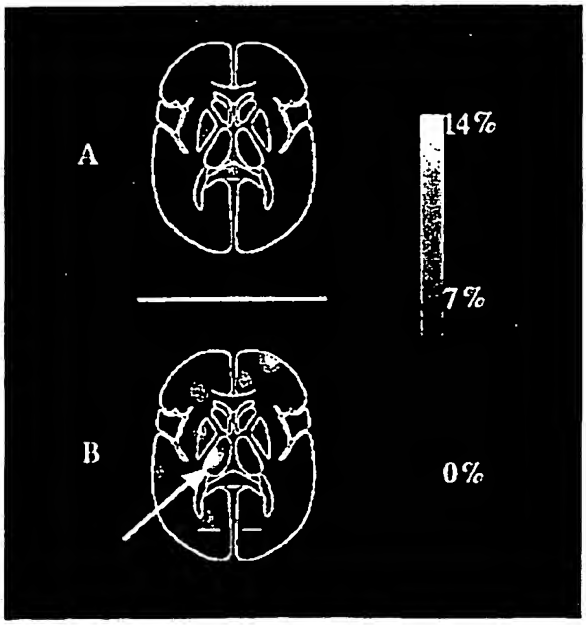


FIG. 1. Composite subtraction PET images (post-levodopa scans minus pre-levodopa scans) shown in transverse orientation, Talairach z level = 6. (*A*) PD patients without DID ($n = 26$). (*B*) PD patients with DID ($n = 6$). Arrow indicates significant thalamic response in this group.

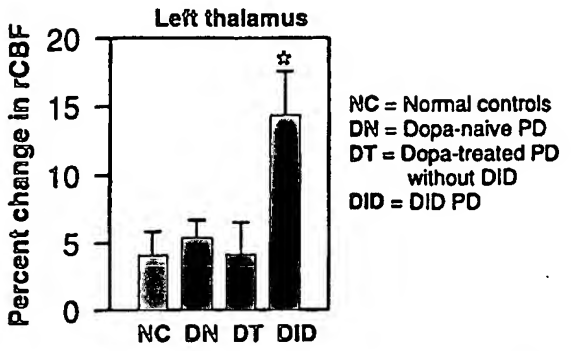


FIG. 2. Mean (± SEM) change in blood flow after levodopa administration for the left thalamus in each group. A group × drug interaction was found only for the left thalamus activation (see *Results* for details). *, Significantly greater increase in blood flow compared with all other groups.

DID group only (left = 14% increase, right = 4% increase, P = 0.04, two-tailed).

2. Main effects of drug were found for all 5 regions (Ps <0.01), indicating significantly increased blood flow after levodopa administration, collapsing across groups, in all regions.

3. There were no main effects of group on overall flow for any region, indicating that there were no systematic differences between groups, collapsing across condition (pre- and post-levodopa), in these regions.

Relationship Between Clinical Variables and Regional Responses. There were no significant correlations between regional blood flow changes in PD patients and symptom duration or change in UPDRS scores after levodopa administration. To examine the effect of symptom and treatment duration on the thalamic response in the PD subgroups more stringently, we included these variables as covariates in repeated measures general linear analyses. The results demonstrated that the interaction between drug (pre- vs. post-levodopa) and PD group for the left thalamic response was still significant (Ps <0.05). Thus, differences between PD subgroups in duration of symptoms or duration of dopa-treatment could not explain differences between PD subgroups in the thalamic response to levodopa.

Clinical Response and Levodopa and Carbidopa Plasma Levels. Levodopa and carbidopa plasma concentrations were measured in 34 subjects. Mean levodopa concentrations peaked between 30 and 60 min after taking levodopa and then remained above levels found to provide symptomatic benefit in other studies (35) during the post-levodopa scans. PD patients demonstrated significant clinical improvement after levodopa administration at the time of the post-levodopa scans, as measured by the modified UPDRS scale (t test, $P < 0.001$), providing further evidence of the therapeutic efficacy of these levodopa levels in our sample. Carbidopa concentrations remained stable across the study. There was no overall effect of group on levodopa levels (P = 0.15).

Relationship Between Levodopa Plasma Levels and Regional Responses. Pearson correlation coefficients were calculated between the five regional responses and levodopa plasma levels at the time of the post-levodopa scans for all subjects. These correlations were not significant.

Global Blood Flow. Although we analyzed all regional data by using normalized PET counts, which are linearly related to quantitative regional cerebral blood flow (31), we also quantified absolute global blood flow in 25 subjects. Global blood flow values did not change significantly after levodopa administration (pre-levodopa mean flow = 59.6 ml/min × 100 g, SD = 15.3; post-levodopa mean flow = 57.6 ml/min × 100 g, SD = 12.8; paired t test, P = 0.24), consistent with other published reports (36, 37).

DISCUSSION

We found that DID patients had large responses in the left thalamus after a dose of levodopa, distinguishing them from all other groups. Responses in the other regions examined did not discriminate DID patients from other PD patients or normals. The striking difference in thalamic response between DID patients and all other groups may represent a significant abnormality in the neurophysiological response of the basal ganglia-thalamocortical circuit to levodopa in patients with DID. We hypothesize that, in DID patients, levodopa causes the inhibitory output of GPi neurons to increase, causing increased flow in the thalamus over the axon termini of GPi neurons (6, 38).

The thalamic response in the DID patients was centered between the left ventroposterolateral and ventrolateral nuclei but likely also involves neighboring nuclei. The GPi and the substantia nigra, pars reticulata (SNpr) have major γ-aminobutyric acid projections to the ventrolateral and ventral anterior nuclei, which in turn provide glutamatergic input to motor, premotor, supplementary motor, and prefrontal cortex (4). The asymmetry of this thalamic response in DID may reflect greater PD manifestations and dyskinesias on the right side of the body in five of the six patients, although all had some bilateral symptoms. The sixth patient had relatively symmetrical involvement at baseline clinical evaluation and was not different from the other patients in the degree of thalamic response to levodopa. Thus, the left lateralization of the thalamic response may indicate, at least in part, the lateralization of symptoms.

Our results and interpretation contradict the common notion that levodopa reduces inhibitory output from GPi to thalamus, leading to increased excitatory output from thalamus to cortex, producing involuntary movements in patients with DID (5). Two types of animal studies support the prevailing model. First, a lesion in the subthalamic nucleus (STN) causes involuntary movements such as hemiballismus and chorea. A STN lesion also causes reduced activity of GPi/SNpr neurons, suggesting that targets of GPi/SNpr neurons, such as thalamus, may have reduced inhibitory input, triggering involuntary movements (39). Second, monkeys with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonism that have developed DID after chronic levodopa treatment have abnormally high glucose uptake in STN. The increased glucose uptake in STN could reflect increased inhibitory input from the external segment of the globus pallidus, which would subsequently reduce its excitatory input to GPi/SNpr (40). Thus, GPi/SNpr neurons may have reduced activity, and consequently reduce their inhibitory action on thalamus, producing dyskinesias. However, in this study (40), glucose uptake was measured while these animals were actively dyskinetic; and feedback from the movements could confound interpretation of the results. Specifically, increased glucose uptake in STN also could reflect increased excitatory input directly from cortex to STN, which might be expected to occur during movements (41).

There are some inconsistencies with this model. This model predicts that pallidotomy would increase DID; whereas, widespread clinical observations demonstrate that pallidotomy consistently reduces DID. If this commonly accepted model were correct, we would have expected a larger decrease in thalamic blood flow in the DID patients compared with non-DID patients after levodopa administration. In addition, Matsumura *et al.* (42) reported that in monkeys who were made dyskinetic by injection of the γ-aminobutyric acid antagonist bicuculline into the external segment of the globus pallidus, neuronal firing increased in many GPi neurons just

before the onset of dyskinesias. They also found an abnormal pattern of firing characterized by bursts and pauses in the external segment of the globus pallidus and GPi neurons after injection. These changes in GPi firing associated with dyskinesias are not consistent with the current models. On the other hand, Hutchison *et al.* (43) reported that the average firing rate in GPi neurons decreased after s.c. apomorphine administration in PD patients undergoing pallidotomy surgery; some of whom experienced dyskinesias. However, no direct comparisons were performed between patients with and without DID. In addition, no changes in firing patterns were reported. Therefore, it is unclear from that study whether GPi neurons increase or decrease firing in response to dopamine agonists.

Two possible mechanisms could explain our finding of increased thalamic blood flow in DID patients after levodopa administration: (*i*) increased inhibitory input from GPi to thalamus or (*ii*) increased excitatory input from cortex to thalamus. To distinguish between these possibilities, we performed an exploratory analysis of our data examining the effect of levodopa on blood flow in motor cortex. If there were increased inhibitory input to thalamus, then thalamic output to motor cortex would be reduced and blood flow there also would be reduced. If there were increased excitatory input from cortex to thalamus, then flow in the motor cortex would be either increased or unchanged. Our images adequately sampled the arm/hand of motor cortex as defined by coordinates from a previous PET study (44). Left and right motor cortex regions were analyzed as previously described for other regions. DID subjects had a significant decrease in left primary motor cortex blood flow, whereas the rest of the groups had either increases in flow (dopa-naïve PD patients, normals) or no change (dopa-treated PD patients without DID). No significant change was found in the right motor cortex for any of these groups (see Fig. 3). These results are internally consistent with the interpretation that the significantly increased thalamic blood flow found in DID subjects is related to increased inhibitory input from GPi. In further support of this idea, we found a significant inverse correlation between left thalamus and left primary motor cortex in all PD subjects ($n = 32$; $r = -0.39$, $P < 0.014$, one-tailed), as predicted.

Another way to address these hypotheses is to examine the thalamic response to levodopa in DID patients before and after pallidotomy surgery. If the thalamic response to levodopa is due to alteration in GPi firing, pallidotomy should reduce the augmented thalamic blood flow response. Such a finding would indicate that the increased thalamic response in DID patients is mediated via output from the basal ganglia rather than direct cortical-thalamic input. Thus, the results of this present study provide a basis for generating specific hypotheses about the mechanisms of DID and the mechanisms by which pallidotomy reduces DID.

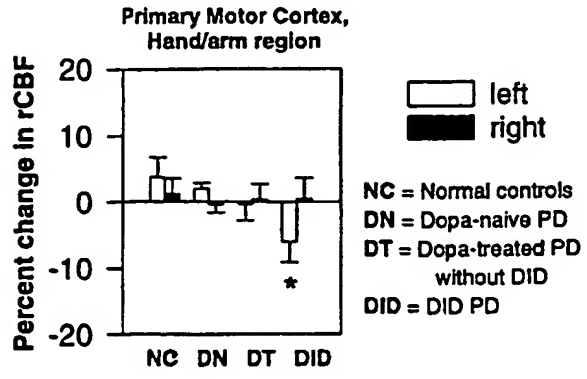


FIG. 3. Mean (± SD) change in blood flow after levodopa administration for left and right primary motor cortex (hand/arm region) for each group. *, Significant difference compared with normal controls and dopa-naïve PD patients.

CONCLUSIONS

In conclusion, we found significantly greater thalamic response to levodopa in PD patients with DID than in PD patients without DID or normals. This difference raises substantial questions about our understanding of how DID are mediated in the brain. Our findings are not consistent with the prevailing hypothesis that DID are mediated by decreased inhibitory output from GPi to thalamus after levodopa administration. However, our results are consistent with the finding that pallidotomy typically reduces DID (3), which is not easily explained by current theory. We hypothesize that levodopa produces elevated blood flow response in the thalamus in patients with DID and that this reflects an alteration in function of neurons projecting from GPi to thalamus. This hypothesis may be tested by comparing dopa-induced blood flow responses in the thalamus of patients with DID before and after pallidotomy. The results of such a test may help to explain the clinical response to pallidotomy.

We thank Lee Tempel, M.D., Ann-Mary MacLeod, and the members of the Division of Radiological Sciences for expert technical assistance. We also thank Marcus E. Raichle, M.D., Amy J. Bastian, P.T., Ph.D., and Jonathan W. Mink, M.D., Ph.D. for helpful comments. This work has been supported by National Institutes of Health Grants NS31001, NS32318, and NS01898, National Institute of Mental Health Grant MH-17104, as well as by the American Parkinson's Disease Association, National Alliance for Research on Schizophrenia and Depression, the Clinical Hypotheses Research Section of the Charles A. Dana Foundation, and the McDonnell Center for the Study of Higher Brain Function.

1. Hornykiewicz, O. (1963) *Wien. Klin. Wochenschr.* 75, 309–312.
2. Cotzias, G. C., van Woert, H. H. & Schiffer, L. M. (1967) *N. Engl. J. Med.* 276, 374–379.
3. Vitek, J. L. & Bakay, R. A. E. (1997) *Curr. Opin. Neurol.* 10, 332–339.
4. Mink, J. W. (1996) *Prog. Neurobiol.* 50, 381–425.
5. Calon, F., Goulet, M., Blanchet, P. J., Martel, J. C., Piercey, M. F., Bedard, P. J. & Paolo, T. D. (1995) *Brain Res.* 680, 43–52.
6. Schwartz, W., Smith, C., Davidsen, L., Savaki, H. & Sokoloff, L. (1979) *Science* 205, 723–725.
7. Black, K. J., Gado, M. H. & Perlmutter, J. S. (1997) *J. Neurosci.* 17, 3168–3177.
8. Engber, T. M., Anderson, J. J., Bolry, R., Kuo, S. & Chase, T. N. (1993) *Neuroscience* 54, 1051–1061.
9. Grasby, P., Frith, C., Paulesu, E., Friston, K., Frackowiak, R. & Dolan, R. (1995) *Exp. Brain Res.* 104, 337–348.
10. McCulloch, J., Savaki, H. E., McCulloch, M. C. & Sokoloff, L. (1980) *Science* 207, 313–315.
11. McCulloch, J., Savaki, H. E., McCulloch, M. C., Jehle, J. & Sokoloff, L. (1982) *Brain Res.* 243, 67–80.
12. Morelli, M., Pontieri, F. E., Linfante, I., Orzi, F. & Di Chiara, G. (1993) *Synapse* 13, 264–269.
13. Trugman, J. M. & Wooten, G. F. (1986) *Brain Res.* 379, 264–274.
14. Trugman, J. M. & Wooten, G. F. (1987) *J. Neurosci.* 7, 2927–2935.
15. Wooten, G. F. (1987) *J. Neurosci.* 7, 2927–2935.
16. The Parkinson's Study Group (1989) *Arch. Neurol.* 46, 1052–1060.
17. Folstein, M. F., Folstein, S. E. & McHugh, P. R. (1975) *J. Psychiatr. Res.* 12, 189–198.
18. Hamilton, A. (1960) *J. Neurol. Neurosurg. Psychiatry* 23, 56–62.
19. Oldfield, R. C. (1971) *Neuropsychologia* 9, 97–113.
20. The Parkinson's Study Group (1996) *Ann. Neurol.* 39, 37–45.
21. Lang, A. E. & Fahn, S. (1989) in *Quantification of Neurologic Deficit*, ed. Munsat, T. (Butterworths, Boston), pp. 285–309.
22. Fox, P. T., Perlmutter, J. S. & Raichle, M. E. (1985) *J. Comput. Assist. Tomogr.* 9, 141–153.
23. Herscovitch, P., Markham, J. & Raichle, M. (1983) *J. Nucl. Med.* 24, 782–789.

24. Spinks, T. J., Jones, T., Bailey, D. L., Townsend, D. W. Q., Grootoonk, S., Bloomfield, P. M., Gilardi, M.-C., Casey, M. E., Sipe, B. & Reed, J. (1992) *Phys. Med. Biol.* 37, 1637–1655.
25. Raichle, M., Martin, W., Herscovitch, P., Mintun, M. & Markham, J. (1983) *J. Nucl. Med.* 24, 790–798.
26. Videen, T. O., Perlmutter, J. S., Herscovitch, P. & Raichle, M. E. (1987) *J. Cereb. Blood Flow Metab.* 7, 513–516.
27. Baruzzi, A., Congin, M., Albani, F. & Riva, J. (1986) *J. Chromatogr.* 375, 165–169.
28. Rondelli, I., Acerbi, D., Mariotti, F. & Ventrua, P. (1994) *J. Chromatogr.* 6, 17–23.
29. Woods, R. P., Mazziotta, J. C. & Cherry, S. R. (1993) *J. Comput. Assist. Tomogr.* 14, 536–546.
30. Talairach, J. & Tournoux, P. (1988) *Co-Planar Stereotaxic Atlas of the Human Brain* (Thieme, New York).
31. Fox, P. & Raichle, M. (1989) *J. Cereb. Blood Flow Metab.* 9, 96–103.
32. Burton, H., MacLeod, A., Videen, T. & Raichle, M. (1997) *Cereb. Cortex* 7, 3–17.
33. Drevets, W., Videen, T., Price, J., Preskorn, S., Carmichael, T. & Raichle, M. (1992) *J. Neurosci.* 12, 3628–3641.
34. Mintun, M., Fox, P. & Raichle, M. (1989) *J. Cereb. Blood Flow Metab.* 9, 96–103.
35. Juncos, J. L., Mouradian, M. M., Fabbrini, G. & Chase, T. N. (1990) in *Therapy of Parkinson's Disease*, eds. Koller W. C. & Paulson G. (Dekker, New York), pp. 185–203.
36. Perlmutter, J. S. & Raichle, M. E. (1985) *Neurology* 35, 1127–1134.
37. Henriksen, L. & Boas, J. (1985) *Acta Neurol. Scand.* 71, 257–266.
38. Ackerman, R., Finch, D., Babb, T. & J Engel, J. (1984) *J. Neurosci.* 4, 251–264.
39. Hamada, I. & DeLong, M. (1992) *J. Neurophysiol.* 68, 1850–1858.
40. Mitchell, I., Boyce, S., Sambrook, M. & Crossman, A. (1992) *Brain* 115, 809–824.
41. Fujimoto, K. & Kita, H. (1993) *Brain Res.* 609, 185–192.
42. Matsumura, M., Tremblay, L., Richard, H. & Filion, M. (1995) *Neuroscience* 65, 59–70.
43. Hutchison, W. D., Levy, R., Dostrovsky, J. O., Lozano, A. M. & Lang, A. E. (1997) *Ann. Neurol.* 42, 767–775.
44. Tempel, L. W. & Perlmutter, J. S. (1992) *J. Cereb. Blood Flow Metab.* 12, 554–561.

STALEVO™ LABELING

STALEVO™ 50

STALEVO™ 100

STALEVO™ 150

(carbidopa, levodopa and entacapone) Tablets

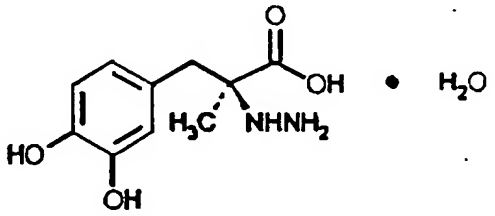
Rx only

Prescribing Information

DESCRIPTION

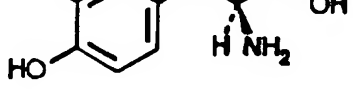
STALEVO™ (carbidopa, levodopa and entacapone) is a combination of carbidopa, levodopa and entacapone for the treatment of Parkinson's disease.

Carbidopa, an inhibitor of aromatic amino acid decarboxylation, is a white, crystalline compound, slightly soluble in water, with a molecular weight of 244.3. It is designated chemically as (-)-L-(α-hydrazino-(α-methyl-β-(3,4-dihydroxybenzene) propanoic acid monohydrate. Its empirical formula is $C_{10}H_{14}N_2O_4 \cdot H_2O$, and its structural formula is

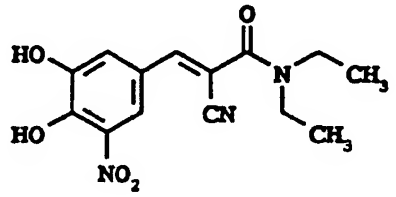


Tablet content is expressed in terms of anhydrous carbidopa, which has a molecular weight of 226.3.

Levodopa, an aromatic amino acid, is a white, crystalline compound, slightly soluble in water, with a molecular weight of 197.2. It is designated chemically as (-)-L-α-amino-β-(3,4-dihydroxybenzene) propanoic acid. Its empirical formula is $C_9H_{11}NO_4$, and its structural formula is



Entacapone, an inhibitor of catechol-*O*-methyltransferase (COMT), is a nitro-catechol-structured compound with a molecular weight of 305.3. The chemical name of entacapone is (E)-2-cyano-3-(3,4-dihydroxy-5-nitrophenyl)-N,N-diethyl-2-propenamide. Its empirical formula is $C_{14}H_{15}N_3O_5$ and its structural formula is



STALEVO™ (carbidopa, levodopa and entacapone) is supplied as tablets in three strengths:

STALEVO 50, containing 12.5mg of carbidopa, 50mg of levodopa and 200mg of entacapone

STALEVO 100, containing 25mg of carbidopa, 100mg of levodopa and 200mg of entacapone

STALEVO 150, containing 37.5mg of carbidopa, 150mg of levodopa and 200mg of entacapone

The inactive ingredients of the STALEVO tablet are corn starch, croscarmellose sodium, glycerol 85%, hypromellose, magnesium stearate, mannitol, polysorbate 80, povidone, sucrose, red iron oxide, titanium dioxide, and yellow iron oxide.

CLINICAL PHARMACOLOGY

Parkinson's disease is a progressive, neurodegenerative disorder of the extrapyramidal nervous system affecting the mobility and control of the skeletal muscular system. Its characteristic features include resting tremor, rigidity, and bradykinetic movements.

Mechanism of Action

Levodopa

Current evidence indicates that symptoms of Parkinson's disease are related to depletion of dopamine in the corpus striatum. Administration of dopamine is ineffective in the treatment of Parkinson's disease apparently because it does not cross the blood-brain barrier. However, levodopa, the metabolic precursor of dopamine, does cross the blood-brain barrier, and presumably is converted to dopamine in the brain. This is thought to be the mechanism whereby levodopa relieves symptoms of Parkinson's disease.

Carbidopa

When levodopa is administered orally it is rapidly decarboxylated to dopamine in extracerebral tissues so that only a small portion of a given dose is transported unchanged to

the central nervous system. Carbidopa inhibits the decarboxylation of peripheral levodopa, making more levodopa available for transport to the brain. When coadministered with levodopa, carbidopa increases plasma levels of levodopa and reduces the amount of levodopa required to produce a given response by about 75 %. Carbidopa prolongs the plasma half-life of levodopa from 50 minutes to 1.5 hours and decreases plasma and urinary dopamine and its major metabolite, homovanillic acid. The t_{max} of levodopa, however, was unaffected by the coadministration.

Entacapone

Entacapone is a selective and reversible inhibitor of catechol-O-methyltransferase (COMT).

In mammals, COMT is distributed throughout various organs with the highest activities in the liver and kidney. COMT also occurs in neuronal tissues, especially in glial cells. COMT catalyzes the transfer of the methyl group of S-adenosyl-L-methionine to the phenolic group of substrates that contain a catechol structure. Physiological substrates of COMT include DOPA, catecholamines (dopamine, norepinephrine, and epinephrine) and their hydroxylated metabolites. The function of COMT is the elimination of biologically active catechols and some other hydroxylated metabolites. When decarboxylation of levodopa is prevented by carbidopa, COMT becomes the major metabolizing enzyme for levodopa, catalyzing its metabolism to 3-methoxy-4-hydroxy-L-phenylalanine (3-OMD).

When entacapone is given in conjunction with levodopa and carbidopa, plasma levels of levodopa are greater and more sustained than after administration of levodopa and carbidopa alone. It is believed that at a given frequency of levodopa administration, these more sustained plasma levels of levodopa result in more constant dopaminergic stimulation in the brain, leading to greater effects on the signs and symptoms of Parkinson's disease. The higher levodopa levels may also lead to increased levodopa adverse effects, sometimes requiring a decrease in the dose of levodopa.

When 200 mg entacapone is coadministered with levodopa/carbidopa, it increases levodopa plasma exposure (AUC) by 35-40% and prolongs its elimination half-life in Parkinson's disease patients from 1.3 to 2.4 hours. Plasma levels of the major COMT-mediated dopamine metabolite, 3-methoxy-4-hydroxy-L-phenylalanine (3-OMD), are also markedly decreased proportionally with increasing dose of entacapone.

In animals, while entacapone enters the CNS to a minimal extent, it has been shown to inhibit central COMT activity. In humans, entacapone inhibits the COMT enzyme in peripheral tissues. The effects of entacapone on central COMT activity in humans have not been studied.

Pharmacokinetics

The pharmacokinetics of Stalevo tablets have been studied in healthy subjects (age 45-75 years old). Overall, following administration of corresponding doses of levodopa, carbidopa and

entacapone as STALEVO or as carbidopa/levodopa product plus Comtan® (entacapone) tablets, the mean plasma concentrations of levodopa, carbidopa, and entacapone are comparable.

Absorption/Distribution:
Both levodopa and entacapone are rapidly absorbed and eliminated, and their distribution volume is moderately small. Carbidopa is absorbed and eliminated slightly more slowly compared with levodopa and entacapone. There are substantial inter- and intra-individual variations in the absorption of levodopa, carbidopa and entacapone, particularly concerning its C_{max}.

The food-effect on the STALEVO tablet has not been evaluated.

Levodopa

The pharmacokinetic properties of levodopa following the administration of single dose STALEVO™ (carbidopa, levodopa and entacapone) tablets are summarized in Table 1.

Table 1. Pharmacokinetic characteristics of levodopa with different tablet strengths of STALEVO (mean ±SD)

Tablet strength	$AUC_{0-\infty}$ (ng·h/mL)	C_{max} (ng/mL)	t_{max} (h)
12.5 - 50 - 200 mg	1040 ± 314	470 ± 154	1.1 ± 0.5
25 - 100 - 200 mg	2910 ± 715	975 ± 247	1.4 ± 0.6
37.5 - 150 - 200 mg	3770 ± 1120	1270 ± 329	1.5 ± 0.9

Since levodopa competes with certain amino acids for transport across the gut wall, the absorption of levodopa may be impaired in some patients on a high protein diet. Meals rich in large neutral amino acids may delay and reduce the absorption of levodopa (see PRECAUTIONS).

Levodopa is bound to plasma protein only to a minor extent (about 10-30%).

Carbidopa

Following administration of STALEVO as a single dose to healthy male and female subjects, the peak concentration of carbidopa was reached within 2.5 to 3.4 hours on average. The mean C_{max} ranged from about 40 to 125 ng/ml and the mean AUC from 170 to 700 ng·h/mL, with different STALEVO strengths providing 12.5 mg, 25 mg, or 37.5 mg of carbidopa.

Carbidopa is approximately 36 % bound to plasma protein.

Entacapone

Following administration of STALEVO as a single dose to healthy male and female subjects, the peak concentration of entacapone in plasma was reached within 1.0 to 1.2 hours on average. The mean C_{max} of entacapone was about 1200 ng/mL and the AUC 1250 to 1450 ng·h/mL after administration of different STALEVO strengths all providing 200 mg of entacapone.

The plasma protein binding of entacapone is 98% over the concentration range of 0.4 - 50 µg/mL. Entacapone binds mainly to serum albumin.

Metabolism and Elimination:

Levodopa
The elimination half-life of levodopa, the active moiety of antiparkinsonian activity, was 1.7 hours (range 1.1-3.2 hours).

Levodopa is extensively metabolized to various metabolites. Two major pathways are decarboxylation by dopa decarboxylase (DDC) and O-methylation by catechol-O-methyltransferase (COMT).

Carbidopa
The elimination half-life of carbidopa was on average 1.6 to 2 hours (range 0.7-4.0 hours).

Carbidopa is metabolized to two main metabolites (á-methyl-3-methoxy-4-hydroxyphenylpropionic acid and á-methyl-3,4-dihydroxyphenylpropionic acid). These 2 metabolites are primarily eliminated in the urine unchanged or as glucuronide conjugates. Unchanged carbidopa accounts for 30% of the total urinary excretion.

Entacapone
The elimination half-life of entacapone was on average 0.8 to 1 hours (0.3-4.5 hours).

Entacapone is almost completely metabolized prior to excretion with only a very small amount (0.2% of dose) found unchanged in urine. The main metabolic pathway is isomerization to the cis-isomer, the only active metabolite. Entacapone and the cis-isomer are eliminated in the urine as glucuronide conjugates. The glucuronides account for 95 % of all urinary metabolites (70% as parent- & 25% as cis-isomer- glucuronides). The glucuronide conjugate of the cis-isomer is inactive. After oral administration of a 14C-labeled dose of entacapone, 10% of labeled parent and metabolite is excreted in urine and 90% in feces.

Due to short elimination half-lives, no true accumulation of levodopa or entacapone occurs when they are administered repeatedly.

Special Populations:

Hepatic Impairment:

STALEVO™ (carbidopa, levodopa and entacapone)

While there are no studies on the pharmacokinetics of carbidopa and levodopa in patients with hepatic impairment, STALEVO should be administered cautiously to patients with biliary obstruction or hepatic disease since biliary excretion appears to be the major route of excretion of entacapone and hepatic impairment had a significant effect on the pharmacokinetics of entacapone when 200mg entacapone was administered alone.

Entacapone

Hepatic impairment had a significant effect on the pharmacokinetics of entacapone when 200mg entacapone was administered alone. A single 200mg dose of entacapone, without levodopa/dopa decarboxylase inhibitor coadministration, showed approximately twofold higher AUC and C_{max} values in patients with a history of alcoholism and hepatic impairment (n=10) compared to normal subjects (n=10). All patients had biopsy-proven liver cirrhosis caused by alcohol. According to Child-Pugh grading 7 patients with liver disease had mild hepatic impairment and 3 patients had moderate hepatic impairment. As only about 10% of the entacapone dose is excreted in urine, as parent compound and conjugated glucuronide, biliary excretion appears to be the major route of excretion of this drug. Consequently, STALEVO should be administered with care to patients with biliary obstruction or hepatic disease.

Renal Impairment:

STALEVO™ (carbidopa, levodopa and entacapone):

STALEVO should be administered cautiously to patients with severe renal disease. There are no studies on the pharmacokinetics of levodopa and carbidopa in patients with renal impairment.

Entacapone:

No important effects of renal function on the pharmacokinetics of entacapone were found. The pharmacokinetics of entacapone have been investigated after a single 200-mg entacapone dose, without levodopa-dopa decarboxylase inhibitor coadministration, in a specific renal impairment study. There were three groups: normal subjects (n=7; creatinine clearance >1.12 mL/sec/1.73 m^2), moderate impairment (n=10; creatinine clearance ranging from 0.60 - 0.89 mL/sec/1.73 m^2), and severe impairment (n=7; creatinine clearance ranging from 0.20 - 0.44 mL/sec/1.73 m^2).

Concurrent Diseases

STALEVO should be administered cautiously to patients with biliary obstruction, hepatic disease, severe cardiovascular or pulmonary disease, bronchial asthma, renal, or endocrine disease.

Elderly:
STALEVO tablets have not been studied in Parkinson's disease patients or in healthy volunteers older than 75 years old. In the pharmacokinetics studies conducted in healthy volunteers following single dose of carbidopa/levodopa/entacapone (as STALEVO or as separate carbidopa/levodopa and Comtan tablets):

Levodopa

The AUC of levodopa is significantly (on average 10 - 20%) higher in elderly (60-75 years) than younger subjects (45-60 years). There is no significant difference in the C_{max} of levodopa between younger (45–60 years) and elderly subjects (60–75 years).

Carbidopa

There is no significant difference in the C_{max} and AUC of carbidopa, between younger (45 – 60 years) and elderly subjects (60–75 years).

Entacapone

The AUC of entacapone is significantly (on average, 15%) higher in elderly (60-75 years) than younger subjects (45-60 years). There is no significant difference in the C_{max} of entacapone between younger (45–60 years) and elderly subjects (60–75 years).

Gender:
The bioavailability of levodopa is significantly higher in females when given with or without carbidopa and/or entacapone. Following a single dose of carbidopa, levodopa and entacapone together, either as STALEVO or as separate carbidopa/levodopa and Comtan tablets in healthy volunteers (age range 45-74 years):

Levodopa
The plasma exposure (AUC & C_{max}) of levodopa is significantly higher in females than males (on average, 40% for AUC and 30% for C_{max}). These differences are primarily explained by body weight. Other published literature showed significant gender effect (higher concentrations in females) even after correction for body weight.

Carbidopa:
There is no gender difference in the pharmacokinetics of carbidopa.

Entacapone:
There is no gender difference in the pharmacokinetics of entacapone.

Drug Interactions: See PRECAUTIONS, Drug Interactions

Clinical Studies

Each STALEVO™ (carbidopa, levodopa and entacapone) tablet, provided in three single-dose strengths, contains carbidopa and levodopa in ratio 1:4 and a 200mg dose of entacapone. The three STALEVO tablet strengths have been shown to be bioequivalent to the corresponding doses of standard release carbidopa-levodopa 25-100mg tablets and Comtan 200mg tablets.

The effectiveness of entacapone as an adjunct to levodopa in the treatment of Parkinson's disease was established in three 24-week multicenter, randomized, double blind placebo-controlled trials in patients with Parkinson's disease. In two of these trials, the patients' disease was "fluctuating", i.e., was characterized by documented periods of "On" (periods of relatively good functioning) and "Off" (periods of relatively poor functioning), despite optimum levodopa therapy. There was also a withdrawal period following 6 months of treatment. In the third trial patients were not required to have been experiencing fluctuations. Prior to the controlled part of these trials, patients were stabilized on levodopa for 2 - 4 weeks.

There is limited experience of using entacapone in patients who do not experience fluctuations.

In the first two studies to be described, patients were randomized to receive placebo or entacapone 200mg administered concomitantly with each dose of carbidopa-levodopa (up to 10 times daily, but averaging 4-6 doses per day). The formal double-blind portion of both trials was 6 months long. Patients recorded the time spent in the "On" and "Off" states in home diaries periodically throughout the duration of the trial. In one study, conducted in the Nordic countries, the primary outcome measure was the total mean time spent in the "On" state during an 18-hour diary recorded day (6 a.m. to midnight). In the other study, the primary outcome measure was the proportion of awake time spent over 24 hours in the "On" state.

In addition to the primary outcome measure, the amount of time spent in the "Off" state was evaluated, and patients were also evaluated by subparts of the Unified Parkinson's Disease Rating Scale (UPDRS), a frequently used multi-item rating scale intended to assess mentation (Part I), activities of daily living (Part II), motor function (Part III), complications of therapy (Part IV), and disease staging (Part V & VI); an investigator's and patient's global assessment of clinical condition, a 7-point subjective scale designed to assess global functioning in Parkinson's Disease; and the change in daily carbidopa-levodopa dose.

In one of the studies, 171 patients were randomized in 16 centers in Finland, Norway, Sweden, and Denmark (Nordic study), all of whom received concomitant levodopa plus dopa-decarboxylase inhibitor (either carbidopa-levodopa or benserazide-levodopa). In the second trial, 205 patients were randomized in 17 centers in North America (US and Canada); all patients received concomitant carbidopa-levodopa.

The following tables display the results of these two trials:

Table 2. Nordic Study

	Baseline	Change from Baseline at Month 6*	p-value vs. placebo
Primary Measure from Home Diary (from an 18-hour Diary Day)			
Hours of Awake Time "On"			
Placebo	9.2	+0.1	–
Entacapone	9.3	+1.5	<0.001
Duration of "On" time after first AM dose (hrs)			
Placebo	2.2	0.0	–
Entacapone	2.1	+0.2	<0.05
Secondary Measures from Home Diary (from an 18-hour Diary Day)			
Hours of Awake Time "Off"			
Placebo	5.3	0.0	–
Entacapone	5.5	- 1.3	<0.001
Proportion of Awake Time "On" * (%)**			
Placebo	63.8	+0.6	–
Entacapone	62.7	+9.3	<0.001
Levodopa Total Daily Dose (mg)			
Placebo	705	+14	–
Entacapone	701	- 87	<0.001
Frequency of Levodopa Daily Intakes			
Placebo	6.1	+0.1	–
Entacapone	6.2	- 0.4	<0.001
Other Secondary Measures			
	Baseline	Change from Baseline at Month 6	p-value vs. placebo
Investigator's Global (overall) % Improved**			
Placebo	–	28	–
Entacapone	–	56	<0.01
Patient's Global (overall) % Improved**			
Placebo	–	22	–
Entacapone	–	39	N.S.‡
UPDRS Total			
Placebo	37.4	-1.1	–
Entacapone	38.5	-4.8	<0.01
UPDRS Motor			
Placebo	24.6	-0.7	–
Entacapone	25.5	-3.3	<0.05
UPDRS ADL			
Placebo	11.0	-0.4	–
Entacapone	11.2	-1.8	<0.05

* Mean; the month 6 values represent the average of weeks 8, 16, and 24, by protocol-defined outcome measure

** At least one category change at endpoint.

*** Not an endpoint for this study but primary endpoint in the North American Study.

‡ Not significant.

Table 3. North American Study

	Baseline	Change from Baseline at Month 6	p-value vs. placebo
Primary Measure from Home Diary (for a 24-hour Diary Day)			
Percent of Awake Time "On"			
Placebo	60.8	+2.0	–
Entacapone	60.0	+6.7	<0.05
Secondary Measures from Home Diary (for a 24-hour Diary Day)			
Hours of Awake Time "Off"			
Placebo	6.6	- 0.3	–
Entacapone	6.8	- 1.2	<0.01
Hours of Awake Time "On"			
Placebo	10.3	+ 0.4	–
Entacapone	10.2	+ 1.0	N.S.‡
Levodopa Total Daily Dose (mg)			
Placebo	758	+ 19	–
Entacapone	804	- 93	<0.001
Frequency of Levodopa Daily Intakes			
Placebo	6.0	+ 0.2	–
Entacapone	6.2	0.0	N.S.‡
Other Secondary Measures			
	Baseline	Change from Baseline at Month 6	p-value vs. placebo
Investigator's Global (overall) % Improved**			
Placebo	–	21	–
Entacapone	–	34	<0.05
Patient's Global (overall) % Improved**			
Placebo	–	20	–
Entacapone	–	31	<0.05
UPDRS Total***			
Placebo	35.6	+2.8	–
Entacapone	35.1	-0.6	<0.05
UPDRS Motor***			
Placebo	22.6	+1.2	–
Entacapone	22.0	-0.9	<0.05
UPDRS ADL***			
Placebo	11.7	+1.1	–
Entacapone	11.9	0.0	<0.05

* Mean; the month 6 values represent the average of weeks 8, 16, and 24, by protocol-defined outcome measure.

** At least one category change at endpoint.

*** Score change at endpoint similarly to the Nordic Study.

‡ Not significant.

Effects on "On" time did not differ by age, sex, weight, disease severity at baseline, levodopa dose and concurrent treatment with dopamine agonists or selegiline.

Withdrawal of entacapone: In the North American study, abrupt withdrawal of entacapone, without alteration of the dose of carbidopa-levodopa, resulted in a significant worsening of fluctuations, compared to placebo. In some cases, symptoms were slightly worse than at baseline, but returned to approximately baseline severity within two weeks following levodopa dose increase on average by 80mg. In the Nordic study, similarly, a significant worsening of parkinsonian symptoms was observed after entacapone withdrawal, as assessed two weeks after drug withdrawal. At this phase, the symptoms were approximately at baseline severity following levodopa dose increase by about 50mg.

In the third placebo-controlled trial, a total of 301 patients were randomized in 32 centers in Germany and Austria. In this trial, as in the other two trials, entacapone 200mg was administered with each dose of levodopa/dopa decarboxylase inhibitor (up to 10 times daily) and UPDRS Parts II and III and total daily "On" time were the primary measures of effectiveness. The following results were seen for the primary measures, as well as for some secondary measures:

Table 4. German-Austrian Study

Primary Measures			
	Baseline	Change from Baseline at Month 6	p-value vs. placebo (LOCF)
UPDRS ADL*			
Placebo	12.0	+0.5	–
Entacapone	12.4	-0.4	<0.05
UPDRS Motor*			
Placebo	24.1	+0.1	–
Entacapone	24.9	-2.5	<0.05
Hours of Awake Time "On" (Home diary)**			
Placebo	10.1	+0.5	–
Entacapone	10.2	+1.1	N.S.‡

Secondary Measures			
	Baseline	Change from Baseline at Month 6	p-value vs. placebo
UPDRS Total*			
Placebo	37.7	+0.6	–
Entacapone	39.0	-3.4	<0.05
Percent of Awake Time "On" (Home diary)**			
Placebo	59.8	+3.5	–
Entacapone	62.0	+6.5	N.S.‡
Hours of Awake Time "Off" (Home diary)**			
Placebo	6.8	-0.6	–
Entacapone	6.3	-1.2	0.07
Levodopa Total Daily Dose (mg)*			
Placebo	572	+4	–
Entacapone	566	-35	N.S.‡
Frequency of Levodopa Daily Intake*			
Placebo	5.6	+0.2	–
Entacapone	5.4	0.0	<0.01
Global (overall) % Improved***			
Placebo	–	34	–
Entacapone	–	38	N.S.‡

* Total population; score change at endpoint.

** Fluctuating population, with 5-10 doses; score change at endpoint.

*** Total population; at least one category change at endpoint.

‡ Not significant.

INDICATIONS

STALEVO™ (carbidopa, levodopa and entacapone) is indicated to treat patients with idiopathic Parkinson's disease:

1) To substitute (with equivalent strength of each of the three components) for immediate release cardidopa/levodopa and entacapone previously administered as individual products.

2) To replace immediate release carbidopa/levodopa therapy (without entacapone) when patients experience the signs and symptoms of end-of-dose "wearing-off" (only for patients taking a total daily dose of levodopa of 600mg or less and not experiencing dyskinesias, see DOSAGE AND ADMINISTRATION).

CONTRAINDICATIONS

STALEVO™ (carbidopa, levodopa and entacapone) tablets are contraindicated in patients who have demonstrated hypersensitivity to any component (carbidopa, levodopa, or entacapone) of the drug or its excipients.

Monoamine oxidase (MAO) and COMT are the two major enzyme systems involved in the metabolism of catecholamines. It is theoretically possible, therefore, that the combination of entacapone and a non-selective MAO inhibitor (e.g., phenelzine and tranylcypromine) would result in inhibition of the majority of the pathways responsible for normal catecholamine metabolism. As with carbidopa-levodopa, nonselective monoamine oxidase (MAO) inhibitors are contraindicated for use with STALEVO. These inhibitors must be discontinued at least two weeks prior to initiating therapy with STALEVO. STALEVO may be administered concomitantly with the manufacturer's recommended dose of MAO inhibitors with selectivity for MAO type B (e.g., selegiline HCl) (See PRECAUTIONS, Drug Interactions).

STALEVO is contraindicated in patients with narrow-angle glaucoma.

Because levodopa may activate malignant melanoma, STALEVO should not be used in patients with suspicious, undiagnosed skin lesions or a history of melanoma.

WARNINGS

The addition of carbidopa to levodopa reduces the peripheral effects (nausea, vomiting) due to decarboxylation of levodopa; however, carbidopa does not decrease the adverse reactions due to the central effects of levodopa. Because carbidopa as well as entacapone permits more levodopa to reach the brain and more dopamine to be formed, certain adverse CNS effects, e.g., dyskinesia (involuntary movements) may occur at lower dosages and sooner with levodopa preparations containing carbidopa and entacapone than with levodopa alone.

The occurrence of dyskinesias may require dosage reduction (see PRECAUTIONS, Dyskinesia).

STALEVO™ (carbidopa, levodopa and entacapone) may cause mental disturbances. These reactions are thought to be due to increased brain dopamine following administration of levodopa. All patients should be observed carefully for the development of depression with concomitant suicidal tendencies. Patients with past or current psychoses should be treated with caution.

STALEVO should be administered cautiously to patients with severe cardiovascular or pulmonary disease, bronchial asthma, renal, hepatic or endocrine disease.

As with levodopa, care should be exercised in administering STALEVO to patients with a history of myocardial infarction who have residual atrial, nodal, or ventricular arrhythmias. In such patients, cardiac function should be monitored carefully during the period of initial dosage adjustment, in a facility with provisions for intensive cardiac care.

As with levodopa, treatment with STALEVO may increase the possibility of upper gastrointestinal hemorrhage in patients with a history of peptic ulcer.

Neuroleptic Malignant Syndrome (NMS): Sporadic cases of a symptom complex resembling NMS have been reported in association with dose reductions or withdrawal of therapy with carbidopa-levodopa. Therefore, patients should be observed carefully when the dosage of STALEVO is reduced abruptly or discontinued, especially if the patient is receiving neuroleptics. NMS is an uncommon but life-threatening syndrome characterized by fever or hyperthermia. Neurological findings, including muscle rigidity, involuntary movements, altered consciousness, mental status changes; other disturbances, such as autonomic dysfunction, tachycardia, tachypnea, sweating, hyper- or hypotension; laboratory findings, such as creatine phosphokinase elevation, leukocytosis, myoglobinuria, and increased serum myoglobin have been reported.

The early diagnosis of this condition is important for the appropriate management of these patients. Considering NMS as a possible diagnosis and ruling out other acute illnesses (e.g., pneumonia, systemic infection, etc.) is essential. This may be especially complex if the clinical presentation includes both serious medical illness and untreated or inadequately treated extrapyramidal signs and symptoms (EPS). Other important considerations in the differential diagnosis include central anticholinergic toxicity, heat stroke, drug fever, and primary central nervous system (CNS) pathology.

The management of NMS should include: 1) intensive symptomatic treatment and medical monitoring and 2) treatment of any concomitant serious medical problems for which specific treatments are available. Dopamine agonists, such as bromocriptine, and muscle relaxants, such as dantrolene, are often used in the treatment of NMS, however, their effectiveness has not been demonstrated in controlled studies.

Drugs Metabolized By Catechol-*O*-Methyltransferase (COMT)

When a single 400mg dose of entacapone was given together with intravenous isoprenaline (isoproterenol) and epinephrine without coadministered levodopa/dopa decarboxylase inhibitor, the overall mean maximal changes in heart rate during infusion were about 50% and 80% higher than with placebo, for isoprenaline and epinephrine, respectively.

Therefore, drugs known to be metabolized by COMT, such as isoproterenol, epinephrine, norepinephrine, dopamine, dobutamine, alpha-methyldopa, apomorphine, isoetherine, and bitolterol should be administered with caution in patients receiving entacapone regardless of the route of administration (including inhalation), as their interaction may result in increased heart rates, possibly arrhythmias, and excessive changes in blood pressure.

Ventricular tachycardia was noted in one 32-year-old healthy male volunteer in an interaction study after epinephrine infusion and oral entacapone administration. Treatment with propranolol was required. A causal relationship to entacapone administration appears probable but cannot be attributed with certainty.

PRECAUTIONS

General

As with levodopa, periodic evaluations of hepatic, hematopoietic, cardiovascular, and renal function are recommended during extended therapy.

Patients with chronic wide-angle glaucoma may be treated cautiously with STALEVO™ (carbidopa, levodopa and entacapone) provided the intraocular pressure is well controlled and the patient is monitored carefully for changes in intraocular pressure during therapy.

Hypotension/Syncope

In the large controlled trials of entacapone, approximately 1.2% and 0.8% of 200mg entacapone and placebo patients treated also with levodopa/dopa decarboxylase inhibitor, respectively, reported at least one episode of syncope. Reports of syncope were generally more frequent in patients in both treatment groups who had an episode of documented hypotension (although the episodes of syncope, obtained by history, were themselves not documented with vital sign measurement).

Diarrhea

In clinical trials of entacapone, diarrhea developed in 60 of 603 (10.0%) and 16 of 400 (4.0%) of patients treated with 200mg of entacapone or placebo in combination with levodopa/dopa decarboxylase inhibitor, respectively. In patients treated with entacapone, diarrhea was generally mild to moderate in severity (8.6%) but was regarded as severe in 1.3%. Diarrhea resulted in withdrawal in 10 of 603 (1.7%) patients, 7 (1.2%) with mild and moderate diarrhea and 3 (0.5%) with severe diarrhea. Diarrhea generally resolved after discontinuation of

entacapone. Two patients with diarrhea were hospitalized. Typically, diarrhea presents within 4 - 12 weeks after entacapone is started, but it may appear as early as the first week and as late as many months after the initiation of treatment.

Hallucinations

Dopaminergic therapy in Parkinson's disease patients has been associated with hallucinations. In clinical trials of entacapone, hallucinations developed in approximately 4.0% of patients treated with 200mg entacapone or placebo in combination with levodopa/dopa decarboxylase inhibitor. Hallucinations led to drug discontinuation and premature withdrawal from clinical trials in 0.8% and 0% of patients treated with 200mg entacapone and placebo, respectively. Hallucinations led to hospitalization in 1.0% and 0.3% of patients in the 200mg entacapone and placebo groups, respectively.

Dyskinesia

Entacapone may potentiate the dopaminergic side effects of levodopa and may therefore cause and/or exacerbate preexisting dyskinesia. Although decreasing the dose of levodopa may ameliorate this side effect, many patients in controlled trials continued to experience frequent dyskinesias despite a reduction in their dose of levodopa. The rates of withdrawal for dyskinesia were 1.5% and 0.8% for 200mg entacapone and placebo, respectively.

Other Events Reported With Dopaminergic Therapy

The events listed below are rare events known to be associated with the use of drugs that increase dopaminergic activity, although they are most often associated with the use of direct dopamine agonists.

Rhabdomyolysis: Cases of severe rhabdomyolysis have been reported with entacapone when used in combination with levodopa. The complicated nature of these cases makes it impossible to determine what role, if any, entacapone played in their pathogenesis. Severe prolonged motor activity including dyskinesia may account for rhabdomyolysis. One case, however, included fever and alteration of consciousness. It is therefore possible that the rhabdomyolysis may be a result of the syndrome described in Hyperpyrexia and Confusion (see PRECAUTIONS, Other Events Reported With Dopaminergic Therapy).

Hyperpyrexia and Confusion: Cases of a symptom complex resembling the neuroleptic malignant syndrome characterized by elevated temperature, muscular rigidity, altered consciousness, and elevated CPK have been reported in association with the rapid dose reduction or withdrawal of other dopaminergic drugs. No cases have been reported following the abrupt withdrawal or dose reduction of entacapone treatment during clinical studies.

Prescribers should exercise caution when discontinuing carbidopa,levodopa andentacapone combination treatment. When considered necessary, withdrawal should proceed slowly. If a decision is made to discontinue treatment with STALEVO, recommendations include monitoring the patient closely and adjusting other dopaminergic treatments as needed. This

syndrome should be considered in the differential diagnosis for any patient who develops a high fever or severe rigidity. Tapering entacapone has not been systematically evaluated.

Fibrotic Complications: Cases of retroperitoneal fibrosis, pulmonary infiltrates, pleural effusion, and pleural thickening have been reported in some patients treated with ergot derived dopaminergic agents. These complications may resolve when the drug is discontinued, but complete resolution does not always occur. Although these adverse events are believed to be related to the ergoline structure of these compounds, whether other, nonergot derived drugs (e.g., entacapone, levodopa) that increase dopaminergic activity can cause them is unknown. It should be noted that the expected incidence of fibrotic complications is so low that even if entacapone caused these complications at rates similar to those attributable to other dopaminergic therapies, it is unlikely that it would have been detected in a cohort of the size exposed to entacapone. Four cases of pulmonary fibrosis were reported during clinical development of entacapone; three of these patients were also treated with pergolide and one with bromocriptine. The duration of treatment with entacapone ranged from 7 - 17 months.

Renal Toxicity

In a one-year toxicity study, entacapone (plasma exposure 20 times that in humans receiving the maximum recommended daily dose of 1600mg) caused an increased incidence of nephrotoxicity in male rats that was characterized by regenerative tubules, thickening of basement membranes, infiltration of mononuclear cells and tubular protein casts. These effects were not associated with changes in clinical chemistry parameters, and there is no established method for monitoring for the possible occurrence of these lesions in humans. Although this toxicity could represent a species-specific effect, there is not yet evidence that this is so.

Hepatic Impairment

Patients with hepatic impairment should be treated with caution. The AUC and C_{max} of entacapone approximately doubled in patients with documented liver disease compared to controls. (See CLINICAL PHARMACOLOGY, Pharmacokinetics, and DOSAGE AND ADMINISTRATION).

Biliary Obstruction

Caution should be exercised when administering STALEVO to patients with biliary obstruction, as entacapone is excreted mostly via the bile.

Information for Patients

The patient should be instructed to take STALEVO™ (carbidopa, levodopa and entacapone) only as prescribed. The patient should be informed that STALEVO is a standard-release formulation of carbidopa-levodopa combined with entacapone that is designed to begin release of ingredients within 30 minutes after ingestion. It is important that STALEVO be

taken at regular intervals according to the schedule outlined by the physician. The patient should be cautioned not to change the prescribed dosage regimen and not to add any additional antiparkinsonian medications, including other carbidopa-levodopa preparations, without first consulting the physician.

Patients should be advised that sometimes a "wearing-off" effect may occur at the end of the dosing interval. The physician should be notified for possible treatment-adjustments if such response poses a problem to patient's every-day life.

Patients should be advised that occasionally, dark color (red, brown, or black) may appear in saliva, urine, or sweat after ingestion of STALEVO. Although the color appears to be clinically insignificant, garments may become discolored.

The patient should be advised that a change in diet to foods that are high in protein may delay the absorption of levodopa and may reduce the amount taken up in the circulation. Excessive acidity also delays stomach emptying, thus delaying the absorption of levodopa. Iron salts (such as in multi-vitamin tablets) may also reduce the amount of levodopa available to the body. The above factors may reduce the clinical effectiveness of the levodopa, carbidopa-levodopa and STALEVO therapy.

NOTE: The suggested advice to patients being treated with STALEVO is intended to aid in the safe and effective use of this medication. It is not a disclosure of all possible adverse or intended effects.

Patients should be informed that hallucinations can occur.

Patients should be advised that they may develop postural (orthostatic) hypotension with or without symptoms such as dizziness, nausea, syncope, and sweating. Hypotension may occur more frequently during initial therapy or when total daily levodopa dosage is increased. Accordingly, patients should be cautioned against rising rapidly after sitting or lying down, especially if they have been doing so for prolonged periods, and especially at the initiation of treatment with STALEVO.

Patients should be advised that they should neither drive a car nor operate other complex machinery until they have gained sufficient experience on STALEVO to gauge whether or not it affects their mental and/or motor performance adversely. Because of the possible additive sedative effects, caution should be used when patients are taking other CNS depressants in combination with STALEVO.

Patients should be informed that nausea may occur, especially at the initiation of treatment with STALEVO.

Patients should be advised of the possibility of an increase in dyskinesia.

Carbidopa-levodopa combination and entacapone are known to affect embryo-fetal development in the rabbit and in the rat, respectively. Accordingly, patients should be advised to notify their physicians if they become pregnant or intend to become pregnant during therapy (see PRECAUTIONS, Pregnancy).

Revised PI 05-22-2003

Carbidopa and entacapone are known to be excreted into maternal milk in rats. Because of the possibility that carbidopa, levodopa and entacapone may be excreted into human maternal milk, patients should be advised to notify their physicians if they intend to breastfeed or are breastfeeding an infant.

Laboratory Tests

Abnormalities in laboratory tests may include elevations of liver function tests such as alkaline phosphatase, SGOT (AST), SGPT (ALT), lactic dehydrogenase, and bilirubin. Abnormalities in blood urea nitrogen and positive Coombs test have also been reported. Commonly, levels of blood urea nitrogen, creatinine, and uric acid are lower during administration of STALEVO than with levodopa.

STALEVO may cause a false-positive reaction for urinary ketone bodies when a test tape is used for determination of ketonuria. This reaction will not be altered by boiling the urine specimen. False-negative tests may result with the use of glucose-oxidase methods of testing for glucosuria.

Cases of falsely diagnosed pheochromocytoma in patients on carbidopa-levodopa therapy have been reported very rarely. Caution should be exercised when interpreting the plasma and urine levels of catecholamines and their metabolites in patients on carbidopa-levodopa therapy.

Entacapone is a chelator of iron. The impact of entacapone on the body's iron stores is unknown; however, a tendency towards decreasing serum iron concentrations was noted in clinical trials. In a controlled clinical study serum ferritin levels (as marker of iron deficiency and subclinical anemia) were not changed with entacapone compared to placebo after one year of treatment and there was no difference in rates of anemia or decreased hemoglobin levels.

Drug Interactions

Caution should be exercised when the following drugs are administered concomitantly with STALEVO™ (carbidopa, levodopa and entacapone).

Anti-hypertensive agents: Symptomatic postural hypotension has occurred when carbidopa-levodopa was added to the treatment of patients receiving antihypertensive drugs. Therefore, when therapy with STALEVO is started, dosage adjustment of the antihypertensive drug may be required.

MAO inhibitors: For patients receiving nonselective MAO inhibitors, see CONTRAINDICATIONS. Concomitant therapy with selegiline and carbidopa-levodopa may be associated with severe orthostatic hypotension not attributable to carbidopa-levodopa alone.

Tricyclic antidepressants: There have been rare reports of adverse reactions, including hypertension and dyskinesia, resulting from the concomitant use of tricyclic antidepressants and carbidopa-levodopa.

Dopamine D2 receptor antagonists (e.g., phenothiazines, butyrophenones, risperidone) and isoniazid: Dopamine D2 receptor antagonists (e.g., phenothiazines, butyrophenones, risperidone) and isoniazid may reduce the therapeutic effects of levodopa.

Phenytoin and papaverine: The beneficial effects of levodopa in Parkinson's disease have been reported to be reversed by phenytoin and papaverine. Patients taking these drugs with carbidopa-levodopa should be carefully observed for loss of therapeutic response.

Iron salts: Iron salts may reduce the bioavailability of levodopa, carbidopa and entacapone. The clinical relevance is unclear.

Metoclopramide: Although metoclopramide may increase the bioavailability of levodopa by increasing gastric emptying, metoclopramide may also adversely affect disease control by its dopamine receptor antagonistic properties.

Drugs known to interfere with biliary excretion, glucuronidation, and intestinal beta-glucuronidase (probenecid, cholestyramine, erythromycin, rifampicin, ampicillin and chloramphenicol): As most entacapone excretion is via the bile, caution should be exercised when drugs known to interfere with biliary excretion, glucuronidation, and intestinal beta-glucuronidase are given concurrently with entacapone. These include probenecid, cholestyramine, and some antibiotics (e.g., erythromycin, rifampicin, ampicillin and chloramphenicol).

Pyridoxine: STALEVO can be given to patients receiving supplemental pyridoxine. Oral coadministration of 10-25mg of pyridoxine hydrochloride (vitamin B6) with levodopa may reverse the effects of levodopa by increasing the rate of aromatic amino acid decarboxylation. Carbidopa inhibits this action of pyridoxine; therefore, STALEVO can be given to patients receiving supplemental pyridoxine.

Effect of levodopa & carbidopa in STALEVO on the metabolism of other drugs: Inhibition or induction effect of levodopa & carbidopa has not been investigated.

Effect of entacapone in STALEVO on the metabolism of other drugs: Entacapone is unlikely to inhibit the metabolism of other drugs that are metabolized by major P450s including CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A. In vitro studies of human CYP enzymes showed that entacapone inhibited the CYP enzymes 1A2, 2A6, 2C9, 2C19, 2D6, 2E1 and 3A only at very high concentrations (IC50 from 200 to over 1000 μM; an oral 200mg dose achieves a highest level of approximately 5 μM in people); these enzymes would therefore not be expected to be inhibited in clinical use. However, no information is available regarding the induction effect from entacapone.

Drugs that are highly protein bound (such as warfarin, salicylic acid, phenylbutazone, and diazepam)

Levodopa
Levodopa is bound to plasma protein only to a minor extent (about 10-30%).

Carbidopa
Carbidopa is approximately 36 % bound to plasma protein.

Entacapone

Entacapone is highly protein bound (98%). In vitro studies have shown no binding displacement between entacapone and other highly bound drugs, such as warfarin, salicylic acid, phenylbutazone, and diazepam.

Hormone levels

Of the ingredients in STALEVO, levodopa is known to depress prolactin secretion and increase growth hormone levels.

Carcinogenesis

In a two-year bioassay of carbidopa-levodopa, no evidence of carcinogenicity was found in rats receiving doses of approximately two times the maximum daily human dose of carbidopa and four times the maximum daily human dose of levodopa.

Two-year carcinogenicity studies of entacapone were conducted in mice and rats. Rats were treated once daily by oral gavage with entacapone doses of 20, 90, or 400mg/kg. An increased incidence of renal tubular adenomas and carcinomas was found in male rats treated with the highest dose of entacapone. Plasma exposures (AUC) associated with this dose were approximately 20 times higher than estimated plasma exposures of humans receiving the maximum recommended daily dose of entacapone (MRDD = 1600mg). Mice were treated once daily by oral gavage with doses of 20, 100 or 600mg/kg of entacapone (0.05, 0.3, and 2 times the MRDD for humans on a mg/m^2 basis). Because of a high incidence of premature mortality in mice receiving the highest dose of entacapone, the mouse study is not an adequate assessment of carcinogenicity. Although no treatment related tumors were observed in animals receiving the lower doses, the carcinogenic potential of entacapone has not been fully evaluated. The carcinogenic potential of entacapone administered in combination with carbidopa-levodopa has not been evaluated.

Mutagenesis

Carbidopa was positive in the Ames test in the presence and absence of metabolic activation, was mutagenic in the *in vitro* mouse lymphoma/thymidine kinase assay in the absence of metabolic activation, and was negative in the *in vivo* mouse micronucleus test.

Entacapone was mutagenic and clastogenic in the *in vitro* mouse lymphoma/thymidine kinase assay in the presence and absence of metabolic activation, and was clastogenic in cultured human lymphocytes in the presence of metabolic activation. Entacapone, either alone or in combination with carbidopa-levodopa, was not clastogenic in the *in vivo* mouse micronucleus test or mutagenic in the bacterial reverse mutation assay (Ames test).

Impairment of Fertility

In reproduction studies with carbidopa-levodopa, no effects on fertility were found in rats receiving doses of approximately two times the maximum daily human dose of carbidopa and four times the maximum daily human dose of levodopa.

Entacapone did not impair fertility or general reproductive performance in rats treated with up to 700mg/kg/day (plasma AUCs 28 times those in humans receiving the MRDD). Delayed mating, but no fertility impairment, was evident in female rats treated with 700mg/kg/day of entacapone.

Pregnancy

Pregnancy Category C. Carbidopa-levodopa caused both visceral and skeletal malformations in rabbits at all doses and ratios of carbidopa-levodopa tested, which ranged from 10 times/5 times the maximum recommended human dose of carbidopa-levodopa to 20 times/10 times the maximum recommended human dose of carbidopa-levodopa. There was a decrease in the number of live pups delivered by rats receiving approximately two times the maximun recommended human dose of carbidopa and approximately five times the maximum recommended human dose of levodopa during organogenesis. No teratogenic effects were observed in mice receiving up to 20 times the maximum recommended human dose of carbidopa-levodopa.

It has been reported from individual cases that levodopa crosses the human placental barrier, enters the fetus, and is metabolized. Carbidopa concentrations in fetal tissue appeared to be minimal.

In embryofetal development studies, entacapone was administered to pregnant animals throughout organogenesis at doses of up to 1000mg/kg/day in rats and 300mg/kg/day in rabbits. Increased incidences of fetal variations were evident in litters from rats treated with the highest dose, in the absence of overt signs of maternal toxicity. The maternal plasma drug exposure (AUC) associated with this dose was approximately 34 times the estimated plasma exposure in humans receiving the maximum recommended daily dose (MRDD) of

1600mg. Increased frequencies of abortions and late/total resorptions and decreased fetal weights were observed in the litters of rabbits treated with maternotoxic doses of 100mg/kg/day (plasma AUCs 0.4 times those in humans receiving the MRDD) or greater. There was no evidence of teratogenicity in these studies.

However, when entacapone was administered to female rats prior to mating and during early gestation, an increased incidence of fetal eye anomalies (macrophthalmia, microphthalmia, anophthalmia) was observed in the litters of dams treated with doses of 160mg/kg/day (plasma AUCs 7 times those in humans receiving the MRDD) or greater, in the absence of maternotoxicity. Administration of up to 700mg/kg/day (plasma AUCs 28 times those in humans receiving the MRDD) to female rats during the latter part of gestation and throughout lactation, produced no evidence of developmental impairment in the offspring.

There is no experience from clinical studies regarding the use of STALEVO in pregnant women. Therefore, STALEVO should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nursing Women

In animal studies, carbidopa and entacapone were excreted into maternal rat milk. It is not known whether entacapone or carbidopa-levodopa are excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when STALEVO is administered to a nursing woman.

Pediatric Use

Safety and effectiveness in pediatric patients have not been established.

ADVERSE REACTIONS

Carbidopa-levodopa

The most common adverse reactions reported with carbidopa-levodopa have included dyskinesias, such as choreiform, dystonic, and other involuntary movements and nausea.

The following other adverse reactions have been reported with carbidopa-levodopa:

Body as a Whole: Chest pain, asthenia.

Cardiovascular: Cardiac irregularities, hypotension, orthostatic effects including orthostatic hypotension, hypertension, syncope, phlebitis, palpitation.

Gastrointestinal: Dark saliva, gastrointestinal bleeding, development of duodenal ulcer, anorexia, vomiting, diarrhea, constipation, dyspepsia, dry mouth, taste alterations.

Hematologic: Agranulocytosis, hemolytic and non-hemolytic anemia, thrombocytopenia, leukopenia.

Hypersensitivity: Angioedema, urticaria, pruritus, Henoch-Schönlein purpura, bullous lesions (including pemphigus-like reactions).

Musculoskeletal: Back pain, shoulder pain, muscle cramps.

Nervous System/Psychiatric: Psychotic episodes including delusions, hallucinations, and paranoid ideation, neuroleptic malignant syndrome (see WARNINGS), bradykinetic episodes ("on-off" phenomenon), confusion, agitation, dizziness, somnolence, dream abnormalities including nightmares, insomnia, paresthesia, headache, depression with or without development of suicidal tendencies, dementia, increased libido. Convulsions also have occurred; however, a causal relationship with carbidopa-levodopa has not been established.

Respiratory: Dyspnea, upper respiratory infection.

Skin: Rash, increased sweating, alopecia, dark sweat.

Urogenital: Urinary tract infection, urinary frequency, dark urine.

Laboratory Tests: Decreased hemoglobin and hematocrit; abnormalities in alkaline phosphatase, SGOT (AST), SGPT (ALT), lactic dehydrogenase, bilirubin, blood urea nitrogen (BUN), Coombs' test; elevated serum glucose; white blood cells, bacteria, and blood in the urine.

Other adverse reactions that have been reported with levodopa alone and with various carbidopa-levodopa formulations, and may occur with STALEVO™ (carbidopa, levodopa and entacapone) are:

Body as a Whole: Abdominal pain and distress, fatigue.

Cardiovascular: Myocardial infarction.

Gastrointestinal: Gastrointestinal pain, dysphagia, sialorrhea, flatulence, bruxism, burning sensation of the tongue, heartburn, hiccups.

Metabolic: Edema, weight gain, weight loss.

Musculoskeletal: Leg pain.

Nervous System/Psychiatric: Ataxia, extrapyramidal disorder, failing, anxiety, gait abnormalities, nervousness, decreased mental acuity, memory impairment, disorientation, euphoria, blepharospasm (which may be taken as an early sign of excess dosage; consideration of dosage reduction may be made at this time), trismus, increased tremor, numbness, muscle twitching, activation of latent Horner's syndrome, peripheral neuropathy.

Respiratory: Pharyngeal pain, cough.

Skin: Malignant melanoma (see also CONTRAINDICATIONS), flushing.

Special Senses: Oculogyric crisis, diplopia, blurred vision, dilated pupils.

Urogenital: Urinary retention, urinary incontinence, priapism.

Miscellaneous: Bizarre breathing patterns, faintness, hoarseness, malaise, hot flashes, sense of stimulation.

Laboratory Tests: Decreased white blood cell count and serum potassium; increased serum creatinine and uric acid; protein and glucose in urine.

Entacapone

The most commonly observed adverse events (>5%) in the double-blind, placebo-controlled trials of entacapone (N=1003) associated with the use of entacapone alone and not seen at an equivalent frequency among the placebo-treated patients were: dyskinesia/hyperkinesia, nausea, urine discoloration, diarrhea, and abdominal pain.

Approximately 14% of the 603 patients given entacapone in the double blind, placebo-controlled trials discontinued treatment due to adverse events compared to 9% of the 400 patients who received placebo. The most frequent causes of discontinuation in decreasing order are: psychiatric reasons (2% vs. 1%), diarrhea (2% vs. 0%), dyskinesia/hyperkinesia (2% vs. 1%), nausea (2% vs. 1%), abdominal pain (1% vs. 0%), and aggravation of Parkinson's disease symptoms (1% vs. 1%).

Adverse Event Incidence in Controlled Clinical Studies of Entacapone

Table 6 lists treatment emergent adverse events that occurred in at least 1% of patients treated with entacapone participating in the double blind, placebo-controlled studies and that were numerically more common in the entacapone group, compared to placebo. In these studies, either entacapone or placebo was added to carbidopa-levodopa (or benserazide-levodopa).

Table 5. Summary of Patients with Adverse Events after Start of Trial Drug Administration. At least 1% in Entacapone Group and > Placebo

SYSTEM ORGAN CLASS Preferred term	Entacapone (n = 603) % of patients	Placebo (n = 400)) % of patients
SKIN AND APPENDAGES DISORDERS		
Sweating increased	2	1
MUSCULOSKELETAL SYSTEM DISORDERS		
Back pain	2	1
CENTRAL & PERIPHERAL NERVOUS SYSTEM DISORDERS		
Dyskinesia	25	15
Hyperkinesia	10	5
Hypokinesia	9	8
Dizziness	8	6
SPECIAL SENSES, OTHER DISORDERS		
Taste Perversion	1	0
PSYCHIATRIC DISORDERS		
Anxiety	2	1
Somnolence	2	0
Agitation	1	0
GASTROINTESTINAL SYSTEM DISORDERS		
Nausea	14	8
Diarrhea	10	4
Abdominal Pain	8	4
Constipation	6	4
Vomiting	4	1
Mouth dry	3	0
Dyspepsia	2	1
Flatulence	2	0
Gastritis	1	0
Gastrointestinal Disorders nos	1	0
RESPIRATORY SYSTEM DISORDERS		
Dyspnea	3	1
PLATELET, BLEEDING & CLOTTING DISORDERS		
Purpura	2	1
URINARY SYSTEM DISORDERS		
Urine Discoloration	10	0
BODY AS A WHOLE - GENERAL DISORDERS		
Back Pain	4	2
Fatigue	6	4
Asthenia	2	1
RESISTANCE MECHANISM DISORDERS		
Infection Bacterial	1	0

The prescriber should be aware that these figures cannot be used to predict the incidence of adverse events in the course of usual medical practice where patient characteristics and other factors differ from those that prevailed in the clinical studies. Similarly, the cited frequencies cannot be compared with figures obtained from other clinical investigations involving different treatments, uses, and investigators. The cited figures do, however, provide the prescriber with some basis for estimating the relative contribution of drug and nondrug factors to the adverse events observed in the population studied.

Effects of gender and age on adverse reactions

No differences were noted in the rate of adverse events attributable to entacapone alone by age or gender.

DRUG ABUSE AND DEPENDENCE

Controlled substance class- STALEVO™ (carbidopa, levodopa and entacapone) is not a controlled substance.

Physical and psychological dependence- STALEVO has not been systematically studied, in animal or humans, for its potential for abuse, tolerance or physical dependence. In premarketing clinical experience, carbidopa-levodopa did not reveal any tendency for a withdrawal syndrome or any drug-seeking behavior. However, there are rare postmarketing reports of abuse and dependence of medications containing levodopa. In general, these reports consist of patients taking increasing doses of medication in order to achieve an euphoric state.

OVERDOSAGE

Management of acute overdosage with STALEVO™ (carbidopa, levodopa and entacapone) is the same as management of acute overdosage with levodopa and entacapone. Pyridoxine is not effective in reversing the actions of STALEVO.

Hospitalization is advised, and general supportive measures should be employed, along with immediate gastric lavage and repeated doses of charcoal over time. This may hasten the elimination of entacapone in particular, by decreasing its absorption/reabsorption from the GI tract. Intravenous fluids should be administered judiciously and an adequate airway maintained.

The adequacy of the respiratory, circulatory and renal systems should be carefully monitored and appropriate supportive measures employed. Electrocardiographic monitoring should be instituted and the patient carefully observed for the development of arrhythmias; if required, appropriate anti-arrhythmic therapy should be given. The possibility that the patient may have taken other drugs, increasing the risk of drug interactions (especially catechol-structured drugs) should be taken into consideration. To date, no experience has been reported with dialysis; hence, its value in overdosage is not known. Hemodialysis or hemoperfusion is unlikely to reduce entacapone levels due to its high binding to plasma proteins.

There are very few cases of overdosage with levodopa reported in the published literature. Based on the limited available information, the acute symptoms of levodopa/dopa decarboxylase inhibitor overdosage can be expected to arise from dopaminergic overstimulation. Doses of a few grams may result in CNS disturbances, with an increasing likelihood of cardiovascular disturbance (e.g. hypotension, tachycardia) and more severe psychiatric problems at higher doses. An isolated report of rhabdomyolysis and another of transient renal insufficiency suggest that levodopa overdosage may give rise to systemic complications, secondary to dopaminergic overstimulation.

There have been no reported cases of either accidental or intentional overdose with entacapone tablets. However, COMT inhibition by entacapone treatment is dose-dependent. A massive overdose of entacapone may theoretically produce a 100% inhibition of the COMT enzyme in people, thereby preventing the O-methylation of endogenous and exogenous catechols.

The highest single dose of entacapone administered to humans was 800mg, resulting in a plasma concentration of 14.1 µg/mL. The highest daily dose given to humans was 2400mg, administered in one study as 400mg six times daily with carbidopa-levodopa for 14 days in 15 Parkinson's disease patients, and in another study as 800mg t.i.d. for 7 days in 8 healthy volunteers. At this daily dose, the peak plasma concentrations of entacapone averaged 2.0 µg/mL (at 45 min., compared to 1.0 and 1.2 µg/mL with 200mg entacapone at 45 min.). Abdominal pain and loose stools were the most commonly observed adverse events during this study. Daily doses as high as 2000mg entacapone have been administered as 200mg 10 times daily with carbidopa-levodopa or benserazide-levodopa for at least 1 year in 10 patients, for at least 2 years in 8 patients and for at least 3 years in 7 patients. Overall, however, clinical experience with daily doses above 1600mg is limited.

The range of lethal plasma concentrations of entacapone based on animal data was 80 - 130 µg/mL in mice. Respiratory difficulties, ataxia, hypoactivity, and convulsions were observed in mice after high oral (gavage) doses.

DOSAGE AND ADMINISTRATION

Individual tablets should not be fractionated and only one tablet should be administered at each dosing interval.

Generally speaking, STALEVO should be used as a substitute for patients already stabilized on equivalent doses of carbidopa-levodopa and entacapone. However, some patients who have been stabilized on a given dose of carbidopa/levodopa may be treated with STALEVO if a decision has been made to add entacapone (see below).

The optimum daily dosage of STALEVO™ (carbidopa, levodopa and entacapone) must be determined by careful titration in each patient. STALEVO tablets are available in three strengths, each in a 1:4 ratio of carbidopa to levodopa and combined with 200mg of entacapone in a standard release formulation (STALEVO 50 containing 12.5mg of carbidopa,

50mg of levodopa and 200mg of entacapone; STALEVO 100 containing 25mg of carbidopa, 100mg of levodopa and 200mg of entacapone; and STALEVO 150 containing 37.5mg of carbidopa, 150mg of levodopa and 200mg of entacapone).

Therapy should be individualized and adjusted according to the desired therapeutic response.

Studies show that peripheral dopa decarboxylase is saturated by carbidopa at approximately 70 to 100mg a day. Patients receiving less than this amount of carbidopa are more likely to experience nausea and vomiting. Experience with total daily dosages of carbidopa greater than 200mg is limited.

Clinical experience with daily doses above 1600mg of entacapone is limited. It is recommended that no more than one STALEVO tablet be taken at each dosing administration. Thus the maximum recommended daily dose of STALEVO is eight tablets per day.

How to transfer patients taking carbidopa-levodopa preparations and Comtan® (entacapone) tablets to STALEVO™ (carbidopa, levodopa and entacapone) tablets

There is no experience in transferring patients currently treated with formulations of carbidopa-levodopa other than immediate release carbidopa-levodopa with a 1:4 ratio (controlled release formulations, or standard release presentations with a 1:10 ratio of carbidopa-levodopa) and entacapone to STALEVO.

Patients who are currently treated with Comtan 200mg tablet with each dose of standard release carbidopa-levodopa, can be directly switched to the corresponding strength of STALEVO containing the same amounts of levodopa and carbidopa. For example, patients receiving one tablet of standard release carbidopa-levodopa 25/100mg and one tablet of Comtan 200mg at each administration can be switched to a single STALEVO 100 tablet (containing 25mg of carbidopa, 100mg of levodopa and 200mg of entacapone).

How to transfer patients not currently treated with Comtan® (entacapone) tablets from carbidopa-levodopa to STALEVO™ (carbidopa, levodopa and entacapone) tablets

In patients with Parkinson's disease who experience the signs and symptoms of end-of-dose "wearing-off" on their current standard release carbidopa-levodopa treatment, clinical experience shows that patients with a history of moderate or severe dyskinesias or taking more than 600mg of levodopa per day are likely to require a reduction in daily levodopa dose when entacapone is added to their treatment. Since dose adjustment of the individual components is impossible with fixed dose products, it is recommended that patients first be titrated individually with a carbidopa-levodopa product (ratio 1:4) and an entacapone product, and then transferred to a corresponding dose of STALEVO once the patient's status has stabilized.

In patients who take a total daily levodopa dose up to 600mg, and who do not have dyskinesias, an attempt can be made to transfer to the corresponding daily dose of STALEVO. Even in these patients, a reduction of carbidopa-levodopa or entacapone may be necessary however, and the provider is reminded that this may not be possible with STALEVO. Since entacapone prolongs and enhances the effects of levodopa, therapy should be individualized and adjusted if necessary according to the desired therapeutic response.

Maintenance of STALEVO treatment

Therapy should be individualized and adjusted for each patient according to the desired therapeutic response.

When less levodopa is required, the total daily dosage of carbidopa-levodopa should be reduced by either decreasing the strength of STALEVO at each administration or by decreasing the frequency of administration by extending the time between doses.

When more levodopa is required, the next higher strength of STALEVO should be taken and/or the frequency of doses should be increased, up to a maximum of 8 times daily and not to exceed the maximum daily dose recommendations as outlined above.

Addition of Other Antiparkinsonian Medications

Standard drugs for Parkinson's disease may be used concomitantly while STALEVO is being administered, although dosage adjustments may be required.

Interruption of Therapy

Sporadic cases of a symptom complex resembling Neuroleptic Malignant Syndrome (NMS) have been associated with dose reductions and withdrawal of levodopa preparations. Patients should be observed carefully if abrupt reduction or discontinuation of STALEVO is required, especially if the patient is receiving neuroleptics. (See WARNINGS.)

If general anesthesia is required, STALEVO may be continued as long as the patient is permitted to take fluids and medication by mouth. If therapy is interrupted temporarily, the patient should be observed for symptoms resembling NMS, and the usual daily dosage may be administered as soon as the patient is able to take oral medication.

Special populations

Patients With Impaired Hepatic Function:

Patients with hepatic impairment should be treated with caution. The AUC and C_{max} of entacapone approximately doubled in patients with documented liver disease, compared to controls. However, these studies were conducted with single-dose entacapone without levodopa/dopa decarboxylase inhibitor coadministration, and therefore the effects of liver disease on the kinetics of chronically administered entacapone have not been evaluated (see CLINICAL PHARMACOLOGY, Pharmacokinetics of Entacapone).

HOW SUPPLIED

STALEVO™ (carbidopa, levodopa and entacapone) is supplied as film-coated tablets for oral administration in the following three strengths:

STALEVO 50 film-coated tablets containing 12.5mg of carbidopa, 50mg of levodopa and 200mg of entacapone.
The round, bi-convex shaped tablets are brownish- or greyish-red, unscored, and embossed "LCE 50" on one side.
HDPE bottle of 100 tablets.....NDC 0078-0407-05
HDPE bottle of 250 tablets.....NDC 0078-0407-28

STALEVO 100 film-coated tablets containing 25mg of carbidopa, 100mg of levodopa and 200mg of entacapone.
The oval-shaped tablets are brownish- or greyish-red, unscored, and embossed "LCE 100" on one side.
HDPE bottle of 100 tablets.....NDC 0078-0408-05
HDPE bottle of 250 tablets.....NDC 0078-0408-28

STALEVO 150 film-coated tablets containing 37.5mg of carbidopa, 150mg of levodopa and 200mg of entacapone
The elongated-ellipse shaped tablets are brownish- or greyish-red, unscored, and embossed "LCE 150" on one side.
HDPE bottle of 100 tablets.....NDC 0078-0409-05
HDPE bottle of 250 tablets.....NDC 0078-0409-28

Store at 25°C (77°F) excursions permitted to 15°-30°C (59°-86°F).
[See USP Controlled Room Temperature.]

STALEVO™ (carbidopa, levodopa and entacapone) tablets are manufactured by Orion Corporation, Orion Pharma (Espoo, Finland) and marketed by Novartis Pharmaceuticals Corporation (East Hanover, N.J. 07936, U.S.A.).

XXXXX-XX

REV: April 2003 Printed in U.S.A. XXXXXXX

British Medical Bulletin (1995) Vol. 51, No. 1, pp.106–122

Gene therapy of lysosomal storage disorders

A Salvetti, J M Heard and O Danos

Retrovirus et Transfert Genetique, Institut Pasteur, Paris, France

Lysosomal storage disorders (LSD) result from deficiencies in enzymes normally implicated in the catabolism of macromolecules inside the lysosome. Many of these enzymes can reach the lysosome after being secreted in the extracellular medium and recaptured by specific cell surface receptors. This has suggested a rationale for therapeutic approaches in LSD, in which the missing enzyme is provided by an external source. Current therapies based on this concept, including the administration of purified enzyme and bone marrow transplantation, have been shown to result in clinical improvements in both animal models and patients. Although considerable difficulties must be surmounted, LSD present a favourable situation for gene therapy. The gene corresponding to the affected enzyme has been identified in most diseases and cDNAs are available. Low and unregulated levels of enzyme activity should be sufficient for correction. Importantly, a variety of gene transfer strategies can be carefully evaluated in animal models.

Most of the catabolism in the living cell takes place in the lysosome. These organelles are formed in the trans Golgi network, from vesicles in which more than 40 different enzymes, mostly acid hydrolases, are selectively packed. The mature lysosome results from a fusion between these enzyme-containing vesicles and late endosomes where macromolecules awaiting disposal are entrapped. The acid pH maintained within the lysosome activates the enzymes, and proteins, nucleic acids and complex sugars are degraded. A deficiency in one of these digestion processes results in the accumulation of the undegraded substrate within the lysosomes, which increase in number and size and can severely impair the physiology of the cell.

More than 30 lysosomal storage disorders (LSD) have been identified which are usually classified according to the undigested macromolecule which accumulates: glycosaminoglycans in mucopolysaccharidoses (MPS), sphingolipids in lipidoses and glycoproteins in glycoproteinoses.[1] Most LSD are due to a failure to synthesize an active form of the relevant enzyme; in some cases, as in the I-cell disease, the defect lies in the inability to target the enzymes to the lysosome (Table). The tissues most affected by the enzyme deficiency are those in which the accumulation of the undigested substrate is the highest. For example, in Krabbe disease the deficiency in galactosylceramidase affects mainly the cells of the central nervous system where the turnover of galactosylceramide is particularly important. In MPS, the missing enzymes are normally implicated in the degradation of glycosaminoglycans which accumulate in spleen, liver, brain and cartilage resulting in bone and joint abnormalities, hepatosplenomegaly, corneal clouding and mental retardation.[2] Similar symptoms are found in patients with Gaucher disease in whom a defect in glucocerebrosidase results in the accumulation of glycosylceramide in monocytes/macrophages.

Many enzymes implicated in LSD are secreted proteins with the notable exception of glucocerebrosidase and acid phosphatase that behave like membrane-associated proteins. In a classic series of experiments, Neufeld and collaborators showed that fibroblasts from MPS patients could be corrected by factors secreted by normal fibroblasts or present in urine concentrates. These 'corrective factors' were identified as the normal enzymes themselves, which were taken up by the mutant cells and targeted to the lysosomes.[1] these enzymes are normally synthesized on membrane-bound polysomes in the rough endoplasmic reticulum and are glycosylated during transit through the endoplasmic reticulum and the Golgi apparatus. There, they are specifically modified by phosphorylation of mannose residues and become ligands for the mannose-6-phosphate receptors (M6PRs). These membrane-anchored receptors cycle between the Golgi compartment, lysosome and the plasma membrane, and direct the phosphorylated enzyme precursors to the organelles, either by selectively packing them into pre-lysosomal vesicles, or by capturing mannose phosphorylated molecules in the extra-cellular environment.[3]

The discovery of this secretion/recapture mechanism has suggested that lysosomal deficiencies could be complemented *in trans* by supplying the missing enzyme either as a purified protein or as a graft of cells secreting the protein. Indeed, in some cases of LSD, treatments involving the infusion of purified enzyme or bone marrow transplantation have demonstrated a therapeutic efficiency in both animal models and patients. The gene corresponding to the affected enzyme has been

Table

Type/syndrome	Enzyme deficiency	Cloned cDNA	Animal models	Affected tissues[a]
MUCOPOLYSACCHARIDOSES				
I/Hurler	α-L-iduronidase	human, canine	dog, cat	CNS, JB, LS
I/Scheie				
II/Hunter	iduronate sulfatase	human	–	CNS, JB, LS
III A/San Filippo A	heparan N-sulfatase	–	–	CNS
III B/San Filippo B	N-acetyl-α-glucosaminidase	–	–	CNS
III C/San Filippo C	acetyl CoA: α-glucosaminide -acetyltransferase	–	–	CNS
III D/San Filippo D	N-acetylglucosamine 6-sulfase	human	goat	CNS
IV A/Morquio A	galactose 6-sulfatase	human	–	JB
IV B/Morquio B	β-galactosidase	human	–	JB
VI/Maroteaux-Lamy	arylsufatase B	human, feline	rat, cat	JB
VII/Sly	β-glucuronidase	human, rat, mouse	mouse, dog	CNS, JB, LS
GLYCORPROTEINOSES				
Fucosidosis	α-L-fucosidase	human	dog	CNS, JB
α-Mannosidosis	α-mannosidase	–	cat, cow	CNS, JB, LS
β-mannosidose	β-mannosidase	–	goat, sheep, cow	CNS
Aspartylglycosaminuria	aspartylglycosaminidase	human	–	CNS, LS
Sialidose	sialidase	–	–	CNS, JB
Galactosialidosis	protective protein	human	dog	CNS, JB, LS

(Table continued on following page)

Table Continued...

Type/syndrome	Enzyme deficiency	Cloned cDNA	Animal models	Affected tissues [a]
LIPIDOSES				
Fabry	α-galactosidase	human	–	kidney
Farber	ceramidase	–	–	JB
Gaucher	glucocerobrosidase	human	mouse	CNS, JB, LS
Krabbe	galactosylceramidase	–	mouse	CNS
GM1 gangliosidosis	β-galactosylceramidase	human	dog, cat	CNS, JB, LS
GM2 gangliosidoses:				
Tay-Sachs	β-hexosaminidase alpha-subunit	human	–	CNS
Sandhoff	β-hexosaminidase, β-subunit	human	cat	CNS
Metachromatic	aryl sulfatase A	human	–	CNS
Leukodystrophy				
Niemann-Pick A and B	sphingomyelinase	human	–	CNS, LS
Niemann-Pick C	–	–	mouse	CNS, LS
OTHER DISORDERS WITH SINGLE ENZYME DEFECT				
Wolman	acid lipase	–	rat	LS
Pompe	α-glucosidase	?	–	Muscle
I-cell disease and	6-phosopho-N-acetylglucosamine	?	–	CNS, JB

Summary of lysosomal storage disorders. [a]Predominantly affected tissues in the most severe forms are indicate (CNS: central nervous system; JB: Joint and bone; LS: liver and spleen)

identified for most LSD and cDNAs are available (Table). Gene transfer represents an interesting alternative approach for the therapy of LSD. It could be used to provide the enzyme *in trans* or to restore the production of a normal enzyme directly in the affected cells. This review describes possible approaches for gene therapy of LSD and discusses their potential as compared to currently available treatments.

DESCRIPTION OF LYSOSOMAL STORAGE DISORDERS

Genetics

The estimated incidence of LSD is approximately 1:10 000 live births. The most prevalent lysosomal disorder is Gaucher's disease with a significantly higher frequency in Ashkenazi Jew population (approximately 1:600 to 1:2500).[4] The same biased incidence has been found for Tay-Sachs disease which is more frequent in Ashkenazi Jews and the French-Canadian populations.

Most LSD share common clinical features, such as mental retardation and abnormal skeletal development. Many of these disorders also cause hepatosplenomegaly which may be the dominant symptom in the milder forms. Within each type of LSD, different forms can be distinguished on the basis of the severity of symptoms and age of onset. The most severe forms appear early in infancy and the disease has a chronic and progressive course leading to death before adulthood. Milder forms can lead to late onset symptoms that do not cause premature death. A property shared by these disorders is the accumulation of undegraded molecules, which may be excreted in the urine and result at the histological level, in the appearance of cells containing enlarged lysosomes or inclusions. The diagnosis of LSD is usually made on fibroblast or leucocyte extracts, using enzyme assays to identify the deficiency.

The gene encoding the normal enzyme has been identified and cloned in several cases (Table) and molecular studies can identify the most common mutations. Analysis of the mutations found in Gaucher, metachromatic leukodystrophy, GM2 gangliosidoses, aspartylglycosaminuria and several MPS, indicates that these diseases are very heterogenous.[1] In some cases, these genetic studies have established a correlation between the genetic lesions and the severity of the disease. For example, the analysis of several MPS I patients has led to the identification of at least 3 common mutations associated with the development of severe forms.[5–7] In Gaucher disease, over 35 different mutations have been documented including missense and nonsense point mutations, splicing mutations, deletions, insertions and a fusion gene. For some of these mutations a correlation was made with the severity of the disease dependent on whether they provoke a complete or partial lack of the enzyme.[8] However, a certain degree of variability exists among patients bearing the same genotype, impairing the reliability of predictions bout the clinical outcome.

Animal models

The characterization of animal models of LSD (Table 1) makes it possible to evaluate the efficiency of new therapeutic approaches. Small laboratory animals like mouse and rats, which can be easily bred on an homogenous genetic background have been described in the case of MPS VI, MPS VII and Krabbe disease.[9–11] Larger animals, like MPS I, MPS VII and fucosidosis dogs or MPS I and MPS VI cats are useful in preclinical studies designed to evaluate the feasibility and efficiency of a gene transfer protocol on a larger scale.[12–16] Many other animal models of LSD have been described,[17–26] but in most cases the deficiency was only documented at the biochemical level without definitive identification of the genetic defect.

New animal models can also be created in mice by knocking out the relevant gene. This method has been used to engineer a model of Gaucher disease by disrupting the glucocerebrosidase gene. Mice homozygous for this mutation have a very low enzyme activity and die early after birth.[27] Although animal models for milder forms of Gaucher disease have to be created for therapeutic experiments, this first model is important for the investigation of the pathogenesis of the most severe forms of this disease.

CURRENT TREATMENTS

Preclinical studies on animal models

Studies on animal models of LSD mainly consist in enzyme replacement therapy through bone marrow transplantation. The rationale for this approach is that the lysosomal enzyme secreted by the engrafted hematopoietic cells will be distributed to different tissues and taken up by deficient cells. In addition, non-deficient cells differentiating toward the monocyte/macrophage lineage probably reduce storage in surrounding deficient cells by degrading glycosaminoglycans accumulated in the local environment. Bone marrow transplantation has been shown to have beneficial effects in MPS I dogs, with a decrease in glycosaminoblycans storage in various tissues including the brain, and a much slower progression of the disease. However, only a slight impact on the evolution of the skeletal deformities was observed.[28,29] A clinical amelioration was also demonstrated in MPS VI cats and in fucosidosis dogs.[30,31] In the latter case, a rapid improvement in the peripheral nerve and visceral lesions as well as a more gradual improvement in the central nervous system pathology were documented. Notably, these experiments illustrate that the effectiveness of this treatment depends

on the age at the time of transplantation. Engraftment at an early age, before the onset of clinical signs reduced the severity and slowed the progression of neurological lesions. Similarly, and effect on skeletal deformities and on brain lysosomal storage was observed only after treatment of neonatal MPS VII mice[32] and of one month-old MPS VII dogs (M Haskins, personal communication). A neurological improvement was also demonstrated in the Twitcher mouse which has a galactocerebrosidase deficiency analogous to Krabbe disease in humans. The increase in galactosylceramidase activity in the brain correlated with the progressive infiltration of donor-derived macrophages.[33] These cells may progressively reduce storage lesions through local enzyme release, cell-to-cell enzyme exchange and phagocytosis of the undigested products. On the other hand, bone marrow transplantation has no effect on the progression of the neurological disease in dogs with GM1 gangliosidosis.[34] The reduction of the neurological lesions observed in some of these experiments is thought to result mainly from the colonization of brain by donor-derived macrophages. However, injection of purified recombinant β-glucuronidase in newborn MPS VII mice have suggested that enzyme molecules can cross the blood-brain barrier when the treatment is initiated very early in life.[35]

Treatment of patients with LSD

The discovery that lysosomal storage in cell culture can be reduced by providing extracellular enzymes has rapidly led to several clinical trials in patients using plasma or cells as sources of enzymes. However, these experiments always resulted in a minimal transient effect.[36]

Allogenic bone marrow transplantation has now been performed on a large number of LSD patients. HLA matched bone marrow transplantation is available to less than half of the patients. Mortalities are 10% and 25% depending whether an HLA-matched relative or an unrelated HLA-matched donor can be found. Biochemical and clinical benefits have been observed in MPS I, MPS II, MPS VI and Gaucher type I patients. Successful engraftment always results in increased enzyme levels in leucocytes and normalization of the liver and spleen sizes. In MPS I and II, a stabilization of skeletal lesions usually occurs, but little improvement of pre-existing lesions is seen. In these cases, severe neurological symptoms appear to be prevented by early transplantation, but definitive conclusions about intellectual development cannot be drawn in the absence of long-term follow up. Successful engraftment can also be effective in mild forms of Krabbe, Niemann-Pick A and metachromatic leukodystrophy, but not in severe cases.[37,38]

Early trials of enzyme infusion conducted in the 1970s on patients affected with Fabry and Gaucher diseases were encouraging.[1] The procedures for large-scale purification of lysosomal enzymes have now been further developed, especially for the treatment of Gaucher's disease. Glucocerebrosidase can be concentrated from human placenta and processed by modifying the oligosaccharide chains, thus exposing the mannose residues necessary for recognition and uptake by macrophages.[39] More than 200 Gaucher patients with the non-neuronopathic form of the disease (type I), have received regular injections of this preparation (Ceredase®). Hematologic recovery, reduction of hepatosplenomegaly and skeletal improvement have been documented.[40,41]

Enzyme therapy could be applied in many other forms of LSD at least as a transient therapy while awaiting a suitable bone marrow donor. However, because of the high cost of the enzyme purification process, this therapy is subject to serious economical constraints.

STRATEGIES FOR GENE THERAPY

Rationale of the approach

The partial success of BMT, which can only be offered to patients with HLA-matched donors, and the economical obstacles associated with enzyme therapy, have stimulated the search for gene therapy approaches. As in the other therapeutic interventions, the goal is to provide tissues with minimal enzyme levels in order to avoid pathological lysosomal storage. Different strategies must be designed according to the nature of the enzyme. A soluble lysosomal enzyme can be distributed to tissues from autologous cells engineered to secrete it into the blood stream. In the case of membrane-associated or membrane-bound enzymes, gene transfer will have to be targeted to the most affected cells.

The cDNAs for nearly 20 human enzymes involved in LSD have been cloned (Table). Some of them have been transfected into COS or CHO cells to overproduce an active enzyme. Some of these studies also demonstrated that the enzyme was secreted in culture medium and that it could be internalized by deficient cells to restore a normal level of enzyme activity. Normal cDNAs have also been introduced in vitro into deficient cells using retroviral vectors and shown to complement the biochemical and phenotypic defect.[42–49]

Gene transfer into hematopoietic cells

Gene transfer into hematopoietic cells can be performed to complement a deficiency affecting the hematopoietic elements themselves, as in the monocyte/macrophage lineage in Gaucher or Niemann-Pick disease, or to reduce lysosomal storage in non-hematopoietic tissues. In this case the stored substrate can be degraded both by the scavenging activity of infiltrating macrophages derived from corrected stem cells and by other cells that have internalized the enzyme secreted by surrounding geneti-

cally modified cells. Recent data also suggest that reduction of storage may also result from cell-to-cell transfer of the lysosomal enzyme.[50,51]

Efficient procedures for retrovirus-mediated gene transfer into hematopoietic stem cells have been developed in the mouse. Donor bone marrow cells are infected in vitro in the presence of fibroblasts producing the retroviral vector and used to reconstitute lethally irradiated syngeneic recipients. If gene transfer occurs into a stem cell with long-term reconstituting capacity, it may be permanently amplified in the peripheral blood differentiated cell population. Hematopoietic chimeras stably expressing a foreign gene in a majority of peripheral cells from all lineages have been obtained.[52]

Several investigators have used retroviral vectors expressing the human glucocerebrosidase cDNA under the control of the viral LTR to demonstrate efficient transduction into murine long-term repopulating marrow cells. Analysis of long-term reconstituted mice (up to 8 months after transplantation) demonstrated the presence of the provirus in bone marrow, spleen and thymus). When bone marrow cells from these animals were transplanted into secondary recipients, the provirus was again detected in various hematopoietic lineages up to 4 months after transplantation. The levels of human glucocerebrosidase activity in bone marrow and spleen macrophages were equal to or greater than the endogenous mouse activity.[53–56] Efficient transduction of the human glucocerebrosidase cDNA was obtained in vitro into a substantial fraction of human hematopoietic progenitor cells from Gaucher patients.[44,57] These studies have encouraged several investigators to plan clinical trials involving gene transfer. However, in the absence of an adequate animal model for Gaucher disease, a therapeutic effect of gene transfer still has to be demonstrated.

A corrective effect of gene transfer into hematopoietic stem cells on lysosomal storage has been shown in 2 studies in MPS VII mice. In the first study, a retroviral vector coding for the rat β-glucuronidase cDNA under the control of a thymidine kinase promoter was used to infect bone marrow cells of two MPS VII mice. The analysis of the treated animals, 6 months after bone marrow transplantation, showed a complete disappearance of lysosomal storage lesions in the liver and spleen.[58] In a second study partial hematopoietic chimeras were obtained using a low irradiation dose conditioning of the recipient animals. Mice with less than 5% hematopoietic cells containing the human β-glucuronidase cDNA under the control of the phosphoglycerate kinase 1 promoter, displayed a complete correction of the liver and spleen, suggesting that small amounts of enzyme delivered locally can be sufficient for correction.[59] This observation is hopeful for clinical application in man, since the current available technology in humans does not provide more than a few percent of genetically-modified cells.

Enzyme delivery into the whole organism by genetically modified cells

In LSD involving a secreted enzyme, any cell type could be chosen as a source, provided that efficient methods for ex vivo gene transfer and stable reimplantation exist. Fibroblasts can be easily obtained from skin biopsies, grown in culture and infected with retroviral vectors. The inclusion of fibroblasts into collagen lattices has been shown to result in the formation of transplantable dermis equivalent.[60] The implantation of these lattices into the peritoneal cavity, mixed with bFGF-coated polytetrafluoroethylene (PTFE) fibers was shown to lead to the rapid formation of individualized neo-organs in which the genetically modified fibroblasts are metabolically active for months. A dense vascularisation of the implants brought the enzyme-secreting fibroblasts in permanent contact with the mesenteric circulation.[61] This procedure has been used to secrete human β-glucuronidase in MPS VII mice after retroviral mediated transfer of the human cDNA into skin primary fibroblasts. The implantation into MPS VII mice of lattices containing fibroblasts secreting the human enzyme was followed by a rapid disappearance of lysosomal storage lesions in the liver and the spleen. Human β-glucuronidase activity was found in the liver, spleen, lung, brain, kidney, heart and bone marrow of the implanted animals.[62] These experiments have shown that engineered fibroblasts, if reimplanted in a suitable environment can provide long-term therapeutic levels of enzyme in an MPS model. The cure was not complete however in the animals which displayed severe skeletal abnormalities when they were treated at the age of 6 to 8 weeks.

Experiments are in progress to test whether implanting enzyme-secreting fibroblasts within the first days of life could facilitate the enzyme access to the developing bones and joints and to the central nervous system.

In the perspective of a clinical trial, the procedure has been scaled up in normal dogs. During follow-up of one year uptake of human β-glucuronidase secreted by neo-organs was demonstrated in liver biopsies, in which the canine enzyme was heat-inactivated (P Moullier, unpublished results).

The skeletal muscle has been proposed as a convenient organ for a systemic delivery of therapeutic proteins.[63] Myoblasts have been isolated from MPS VII dog skeletal muscle, grown in culture and infected with a rat β-glucuronidase cDNA-containing retroviral vector. Enzyme expression was documented in both myoblasts and myotubes.[64]

Myoblasts from adult MPS VII mice were also isolated and infected with a retroviral vector coding for human β-glucuronidase. These cells were then injected in MPS VII mice, following muscle injury. The genetically-modified cells were found to efficiently participate to the constitution of regenerated muscle fiber. However, despite an efficient in vitro secretion of the enzyme, only trace amounts of activity were found in the liver and spleen of the treated animals.[65] This suggested that β-glucuronidase was blocked before it could access the blood stream, possibly at the level the muscle basal membrane or immediately reinternalized through binding to M6PRs which are highly expressed in muscle cells.

The liver occupies a strategic position as a provider of proteins into the blood stream. Retrovirus-mediated gene transfer in situ into the liver has been described in mice, rats and dogs.[66,67]Attempts at transferring the β-glucuronidase cDNA into the liver of MPS VII dogs are currently being made. The first results indicate that the fraction of hepatocyte which can be modified by this procedure may be too small to provide therapeutic enzyme levels.

Enzyme delivery to the central nervous system

It is unlikely that a soluble lysosomal enzyme delivered into the serum will cross the blood-brain barrier under normal conditions.[68] The β-glucuronidase found in the brain of MPS VII mice implanted with fibroblasts secreting the enzyme may correspond to enzyme molecules absorbed by monocytes in the periphery and transported across the barrier.[62] In this case, however, the small amount of enzyme found in this tissue may be too low to obtain a correction of the lysosomal storage lesions.

Crossing of the blood-brain barrier could be achieved by coupling the soluble enzyme to an antibody or a ligand recognized by a receptor present on the surface of endothelial cells. It was shown that when NGF was coupled to an antibody against the transferrin receptor, it could cross the blood-brain barrier after peripheral injection in rats.[69] However, in the case of lysosomal enzymes, fusion molecules may loose their catalytic activity or their ability to be recognized by the M6P receptor. Whether these large molecules can be efficiently transported across the endothelial cells also remains to be demonstrated.

Another possible problem may be that, even if the soluble enzyme can cross the blood-brain barrier, it may not be taken up by the cells that need to be corrected. Indeed, delivery of hexosaminadase A to the brain of GM2 gangliosidosis cats, by reversible blood-brain barrier permeabilization lead to a significative concentration of the enzyme in the brain but no detectable uptake by neurons which are the affected

cells. Targeting of neurons was obtained in vitro only after coupling of hexosaminidase A via disulfide linkage to the atoxic fragment C of tetanus toxin.[70]

An alternative solution would be to install intracerebral implants of genetically-modified fibroblasts and myoblasts.[71,72] In the case of LSD however, enzyme delivery throughout the brain is needed and the modified cells should be able to migrate after implantation. Genetically-modified astroglial (O2A) progenitors can be used to assess the capacity of a limited number of cells scattered in the brain to eliminate lysosomal storage. However, the difficulty to access the target cell for ex vivo gene transfer makes this procedure of little therapeutic relevance. Multipotent immortalized neural progenitor cell lines with high migration capacity have been described in the mouse[73] and used to obtain long-term diffuse engraftment in the brain of newborn MPS VII animals after the transfer of the β-glucuronidase cDNA (J Wolfe, personal communication). The availability of such cells in humans could be of genuine interest for the treatment of CNS lesions in LSD.

Direct gene transfer into the CNS is feasible with herpesvirus or adenovirus vectors. A recombinant HSV-1 virus encoding for the rat β-glucuronidase was used to infect MPS VII mice by corneal inoculation. After several weeks, few positive neurons were detected by histochemical staining in the trigeminal ganglia and brain stem of treated mice.[74] The disappearance of lysosomal storage in or around the positive cells was not studied. Although this has not been tested in LSD models yet, a more potent gene transfer can be obtained with adenovirus, by stereotactic injection of vector particles in the brain tissue or in the ventricular space. This second approach leads to the infection of the ependymal cells lining the ventricule and can be used to secrete a protein in the CSF. In LSD, this could directly reduce the levels of undegraded molecules in the CSF and might help enzyme diffusion to large areas of the brain.

Perspectives for clinical trials

Four gene therapy trials have already been approved for the treatment of Gaucher disease by retroviral-mediated transfer of the human glucocerebrosidase cDNA into hematopoietic stem cells.[75] Human $CD34^+$ cells will be purified from G-CSF-mobilized peripheral blood stem cells or from bone marrow and transduced with retrovirus containing media. The retroviral vectors used express the human glucocerebrosidase cDNA under the control of the viral LTR. The transduced cells will then be infused into the patient. If peripheral blood stem cells are used this procedure can be repeated several times while if bone marrow is used, only one treatment will be done. The aims of these trials are: (i) to

examine the safety and the efficiency of transducing the human glucocerebrosidase into CD34 cells by retrovirus-mediated gene transfer; (ii) to determine the extent of long-term persistence of transduced cells in patients; (iii) to investigate whether the enzyme is expressed efficiently enough to improve the patient conditions. For safety reasons, early trials do not include myeloablative conditioning treatment. It is not known whether a therapeutic effect can be obtained in such conditions, since low levels of engraftment of genetically-modified cells are expected.

Regarding MPS, in vivo gene transfer data have been obtained with MPS VII animals and it would seem logical to consider patients with Sly syndrome as the first candidates for a gene therapy trial. However, these patients are exceedingly rare, with less than 20 known cases of live birth. Hurler disease is one of the more frequent MPS. A genotype/phenotype correlation has begun to be established and pre or perinatal diagnosis is feasible. The mechanisms of synthesis, processing, secretion and uptake of β-glucuronidase and α-L-iduronidase are similar, and it is likely that most of the gene transfer data obtained in MPS VII animals can be extrapolated to MPS I. Analysis of Nude mice implanted with neo-organs secreting the human α-L-iduronidase indicates that the enzyme is internalized in the liver and the spleen as efficiently as β-glucuronidase (A Salvetti, unpublished results). The therapeutic efficacy of the gene therapy approaches defined in MPS VII models can also be tested in MPS I dogs.[12] Two types of intervention on MPS I patients could be proposed in the near future, involving retrovirus-mediated gene transfer to either hematopoietic ($CD34^+$) cells or to skin fibroblasts reimplanted into the peritoneal cavity. The graft of autologous skin fibroblast secreting α-L-iduronidase from vascularised neo-organs could be performed using a minimally invasive surgical procedure. Initial trials will have to assess the feasibility of the procedure, its tolerance by the patient, the efficiency and duration of enzyme secretion and the effect on the course of the disease.

More clinical trials are likely to be organized within the next few years for the treatment of other LSD, and Niemann-Pick A and B or metachromatic leucodystrophy are likely candidates. However, the multiplication of clinical trials will critically depend on the issue of the early ones, which therefore have to be conducted very rigorously. As clinical applications will progress, it will remain essential to perform careful experiments in animal models. The uncommon wealth of animals affected with these diseases provides a unique opportunity to base gene therapy trials on a solid collection of scientific and preclinical data.

REFERENCES

1 Neufeld EF. Lysosomal storage diseases. Annu Rev Biochem 1991; 60: 257–280.
2 Hopwood JJ, Morris CP. The mucopolysaccharidoses: diagnosis, molecular genetics and treatment. Mol Biol Med 1990; 7: 381–404.
3 Pfeffer SR. Targeting of proteins to the lysosome. Curr Top Microbiol Immunol 1991; 170: 43–63.
4 Barranger JA, Ginns EI. Glucosylceramide lipidoses: Gaucher disease. In: Scriver CJ, Beaudet AL, Sly WS and Valle D eds. The metabolic basis of inherited disease. New York: McGraw-Hill 1989; 1677–1698.
5 Scott HS, Litjens T, Nelson PV et al. Identification of mutations in the α-L-iduronidase gene (IDUA) causing Hurler and Scheie syndromes. Am J Hum Genet 1993; 53: 973–986.
6 Scott HS, Litjens T, Nelson PV, Brooks DA, Hopwood JJ, Morris CP. α-L-iduronidase mutations (Q70X and P533R) associate with a severe Hurler phenotype. Human Mutation 1992;1: 333–339.
7 Scott HS, Litjens T, Hopwood JJ, Morris CP. A common mutation for mucopolysaccharidosis type I associated with a severe Hurler syndrome phenotype. Hum Mutation 1992; 1: 103–108.
8 Beutler E. Gaucher disease as a paradigm of current issues regrading single gene mutations of humans. Proc Natl Acad Sci USA 1993; 90: 5384–5390.
9 Yoshida M, Noguchi J, Ikadai H, Takahashi M, Nagase S. Arylsulfatase B-deficient mucopolysaccharidosis in rats. J Clin Invest 1993; 91: 1099–1104.
10 Birkenmeier EH, Davisson MT, Beamer WG et al. Murine mucopolysaccharidosis type VII. Characterization of a mouse with β-glucuronidase deficiency. J Clin Invest 1989; 83: 1258–1266.
11 Kobayashi T, Yamanaka T, Jacobs JM, Teixera F, Suzuki K. The Twicher mouse: an enzymatically authentic model of human globoid cell leukodystrophy (Krabbe disease). Brain Res 1980; 202: 479–483.
12 Shull RM, Munger RJ, Spellacy E, Hall CW, Constantopoulos G, Neufeld E. Canine α-L-iduronidase deficiency: a model of Mucopolysaccharidosis I. Am J Pathol 1982; 109: 244–248.
13 Haskins ME, Desnick RJ, DiFerrante N, Jezyk P, Patterson DF. Beta-glucuronidase deficiency in a dog: a model of mucopolysaccharidosis VII. Pediatr Res 1984; 18: 980–984.
14 Healy PJ, Farrow BRH, Nicholas FW, Hedberg K, Ratcliffe R. Canine fucosidosis: a biochemical and genetic investigation. Res Vet Sci 1984; 36: 354–359.
15 Haskins ME, Jezyk PF, Desnick RJ, McDonoug SK, Patterson DF. Alpha-l-iduronidase deficiency in a cat: a model for mucopolysaccharidosis I. Pediatr Res 1979; 13: 1294–1297.
16 Jezyk PF, Haskins ME, Patterson DF, Mellman WJ, Greenstein M. Mucopolysaccharidosis in a cat with aryl-sulfatase B deficiency: a model of Maroteaux-Lamy syndrome. Science 1977; 198: 834–836.
17 Thompson JN, Jones MZ, Dawson G, Huffman PS. N-acetylglucosamine 6–sulphatase deficiency in a Nubian goat: a model of Sanfilippo syndrome type D (mucopolysaccharidosis IIID). J Inherit Metab Dis 1992; 15: 760–768.
18 Vandevelde M, Faukhauser R, Bichsel P, Weismann V, Herschkowitz N. Hereditary neurovisceral mannosidosis associated with α-mannosidase deficiency in a family of Persian cats. Acta Neuropathol (Berl) 1982; 58: 64–66.
19 Sasaki M, Lovell KL, Moller JR. Myelin-associated glycoprotein (MAG) in myelin deficiency of caprine beta-mannosidosis. Brain Res 1993; 620: 127–132.
20 Pearce RD, Callahan JW, Little PB, Klunder LR, Clarke JT. Caprine beta-D-mannosidosis: characterization of a model lysosomal storage disorder. Can J Vet Res 1990; 54: 22–29.
21 Bryan L, Schmutz S, Hodges SD, Snyder FF. Bovine beta-mannosidosis: pathologic and genetic findings in Salvers calves. Vet Pathol 1993; 30: 130–139.

22 Kaye EM, Alroy J, Raghavan SS et al. Dysmyelinogenesis in animal models of GM1 gangliosidosis. Pediatr Neurol 1992; 8: 255–261.
23 Cork LC, Munnel JF, Lorenz MD, Murphy JV, Baker JH, Rattazzi MC. GM2 ganglioside lysosomal storage in cats with β-hexosaminadase deficiency. Science 1977; 196: 1014–1017.
24 Weintraub H, Abramovici A, Amichai D et al. Morphometric studies of pancreatic acinar granule formation in NCTR-Balb/c mice. J Cell Sci 1992; 102: 141–147.
25 Honda Y, Kuriyama M, Higuchi I, Fujiama J, Yoshida H, Osame M, Muscular involvement in lysosomal acid lipase deficiency in rats. J Neurol Sci 1992; 108: 189–195.
26 Knowles K, Alroy J, Castagnaro M, Raghavan SS, Jakowski RM, Freden GO. Adult-onset lysosomal storage disease in a Scipperke dog: clinical, morphological and biochemical studies. Acta Neuropathol (Berl) 1993; 86: 306–312.
27 Tybulewicz VL, Tremblay ML, LaMarca ME et al. Animal model of Gaucher's disease from targeted disruption of the mouse glucocerebrosidase gene. Nature 1992; 357: 407–410.
28 Shull RM, Walker MA. Radiographic findings in a canine model of Mucopolysaccharidosis I. Invest Radiol 1988; 23: 124130.
29 Shull RM, Hastings NE, Selcer RR et al. Bone marrow transplantation in canine mucopolysaccharidosis I. J Clin Invest 1987; 79: 435–443.
30 Gasper PW, Thrall MA, Wenger DA et al. Correction of feline arylsulfatase B deficiency (mucopolysaccharidosis VI) by bone marrow transplantation. Nature 1984; 312: 467–469.
31 Taylor RM, Farrow BR, Stewart GJ. Amelioration of the clinical disease following bone marrow transplantation in fucosidase-deficient dogs. Am J Med Genet 1992; 53: 628–632.
32 Sands MS, Barker JE, Vogler C et al. Treatment of murine mucopolysaccharidosis type VII by syngeneic bone marrow transplantation in neonates. Lab Invest 1993; 68: 676–686.
33 Hoogerbrugge PM, Suzuki K, Suzuki K et al. Donor-derived cells in the central nervous system of Twitcher mice after bone marrow transplantation. Science 1988; 239: 1035–1038.
34 O'Brien JS, Storb R, Raff et al. Bone marrow transplantation in canine GM1 gangliosidosis. Clin Genet 1990; 38: 274–280.
35 Vogler C, Sands M, Higgins A et al. Enzyme replacement with recombinant β-glucuronidase in the newborn mucopolysaccharidosis type VII mouse. Pediatr Res 1993; 34: 837–840.
36 Neufeld EF, Lim TW, Shapiro LJ, Inherited disorders of lysosomal metabolism. Annu Rev Biochem 1977; 44: 357–376.
37 Krivit W, Shapiro E, Hoogerbrugge PM, Moser HW. State of the art review: bone marrow transplantation treatment for storage disease. Bone Marrow Transplant 1992; 10: 87–97.
38 Hoogerbrugge PM, Brouwer OF, Aubourg P et al. Limited role for allogenic bone marrow transplantation in metabolic diseases. (Submitted 1994)
39 Furbish FS, Steer CJ, Krett NL, Barranger JA. Uptake and distribution of placental glucocerebrosidase in rat hepatic cells and effects of sequential deglycosylation. Biochim Biophys Acta 1981; 673: 425–434.
40 Barton NW, Brady PR, Dambrosia JM et al. Replacement therapy for inherited enzyme deficiency – macrophage-targeted glucocerebrosidase for Gaucher's disease. N Engl J Med 1991; 23: 1464–1470.
41 Pastores GM, Sibille AR, Grabowski GA. Enzyme therapy in Gaucher Disease type I: dosage efficacy and adverse effects in 33 patients treated for 6 to 24 months. Blood 1993; 82: 408–416.
42 Anson DS, Bielicki J, Hopwood JJ. Correction of mucopolysaccharidosis type I fibroblasts by retroviral-mediated transfer of the human α-L-iduronidase gene. Hum Gene Ther 1992; 3: 371–379.

43 Anson DS, Taylor JA, Bielicki J et al. Correction of human mucopolysaccharidosis type-VI fibroblasts with recombinant N-acetylgalactosamine-4-sulphatase. Biochem J 1992; 284: 789–794.
44 Fink JK, Correl PH, Perry LK, Brady RO, Karlsson S. Correction of glucocerbrosidase deficiency after retroviral-mediated gene transfer into hematopoietic progenitor cells from patients with Gaucher disease. Proc Natl Acad Sci USA 1990; 8 7: 2334–2338.
45 Occhiodoro T, Hopwood JJ, Morris CP, Anson DS. Correction of alpha-L-fucosidase deficiency in fucosidosis fibroblasts by retroviral vector-mediated gene transfer. Hum Gene Ther 1993; 3: 365–369.
46 Peters C, Rommerskirch W, Modaressi S, von Figura K. Restoration of arylsulphatase B activity in human mucopolysaccharidosis-type-VI fibroblasts by retroviral-vector-mediated gene transfer. Biochem J 1991; 276; 499–504.
47 Suchi M, Dinur T, Desnick RJ et al. Retroviral-mediated transfer of the human acid phingomyelinase cDNA: correction of the metabolic defect in cultured Niemann-Pick disease cells. Proc Natl Acad Sci USA 1992; 89: 3227–3231.
48 Rommerskirch W, Fluharty AL, Peters C, von Figura K, Gieselman V. Restoration of arylsulfatase A activity in human-metachromatic-leucodystrophy fibroblasts via retroviral-vector mediated gene transfer. Biochem J 1991; 280: 459–461.
49 Wolfe JH, Schuchman EH, Stramm LE et al. Restoration of normal lysosomal function in mucopolysaccharidosis type VII cells by retroviral vector-mediated gene transfer. Proc Natl Acad Sci USA 1990; 87: 2877–2881.
50 Olsen I, Bou-Gharios G, Abraham D, Chain B. Lysosomal enzyme transfer from different types of lymphoid cell. Exp Cell Res 1993; 209: 133–139.
51 Bou-Gharios G, Adams G, Pace P, Warden P, Olsen I. Correction of a lysosomal deficiency by contact-mediated enzyme transfer after bone marrow transplantation. Transplantation 1993; 56: 991–996.
52 Karlsson S. Treatment of genetic defects in hematopoietic cell function by gene transfer. Blood 1991; 78: 2481–2492.
53 Correl PM, Kew Y, Perry LK, Brady RO, Fink JK, Karlsson S. Expression of human glucocerebrosidase in long-term reconstituted mice following retroviral-mediated gene transfer into hematopoietic stem cells. Hum Gene Ther 1990; 1: 227–287.
54 Weinthal J, Nolta JA, Yu XJ, Lilley J, Uribe L, Kohn DB. Expression of human glucocerebrosidase following retroviral-mediated transduction of murine hematopoietic stem cells. Bone Marrow Transplant 1991; 8: 403–412.
55 Correl PM, Colilla S, Dave HPG, Karlsson S. High levels of human glucocerebrosidase activity in macrophages of long-term reconstituted mice after retroviral infection of hematopoietic stem cells. Blood 1992; 80: 311–336.
56 Ohashi T, Boggs S, Robbins P et al. Efficient transfer and sustained high expression of the human glucocerebrosidase gene in mice and their functional macrophages following transplantation of bone marrow transduced by a retroviral vector. Proc Natl Acad Sci USA 1992; 89: 11332–11336.
57 Nolta JA, Yu XJ, Bahner I, Kohn DB. Retroviral mediated transfer of the human glucocerebrosidase gene into cultured Gaucher bone marrow. J Clin Invest 1992; 90: 342–348.
58 Wolfe JH, Sands MS, Barker JE et al. Reversal of pathology in murine mucopolysaccharidosis type VII by somatic cell gene transfer. Nature 1992; 360: 749–753.
59 Marechal V, Naffakh N, Danos O, Heard JM. Disappearance of lysosomal storage in spleen and liver of mucopolysaccharidosis VII mice after transplantation of genetically-modified bone marrow cells. Blood 1993; 82: 1358–1365.
60 Bell E, Ivarsson B, Merrill C. Production of a tissue-like structure by contraction of collagen lattices by human fibroblasts of different proliferative potential in vitro. Proc Natl Acad Sci USA 1979; 76: 1274–1278.
61 Moullier P, Maréchal V, Danos O, Heard JM. Continuous systemic secretion of a lysosomal enzyme by genetically-modified mouse skin fibroblasts. Transplantation 1993; 56: 427–432.

Vol. 281 No. 3, January 20, 1999

Original Contribution

TABLE OF CONTENTS >

Prevalence of Lysosomal Storage Disorders

Peter J. Meikle, PhD; John J. Hopwood, PhD; Alan E. Clague, FRCPN; William F. Carey, PhD

JAMA. 1999;281:249-254.

ABSTRACT

Context Lysosomal storage disorders represent a group of at least 41 genetically distinct, biochemically related, inherited diseases. Individually, these disorders are considered rare, although high prevalence values have been reported in some populations. These disorders are devastating for individuals and their families and result in considerable use of resources from health care systems; however, the magnitude of the problem is not well defined. To date, no comprehensive study has been performed on the prevalence of these disorders as a group.

Objective To determine the prevalence of lysosomal storage disorders individually and as a group in the Australian population.

Design Retrospective case studies.

Setting Australia, from January 1, 1980, through December 31, 1996.

Main Outcome Measure Enzymatic diagnosis of a lysosomal storage disorder.

Results Twenty-seven different lysosomal storage disorders were diagnosed in 545 individuals. prevalence ranged from 1 per 57,000 live births for Gaucher disease to 1 per 4.2 million live birt sialidosis. Eighteen of 27 disorders had more than 10 diagnosed cases. As a group of disorders, combined prevalence was 1 per 7700 live births. There was no significant increase in the rate of clinical diagnoses or prenatal diagnoses of lysosomal storage disorders during the study period.

Conclusions Individually, lysosomal storage disorders are rare genetic diseases. However, as a they are relatively common and represent an important health problem in Australia.

INTRODUCTION

Jump to
- Top
- Introductio
- Methods
- Results
- Comment
- Author infc
- References

Lysosomal storage disorders (LSDs) represent a group of at least 41 distinct genetic diseases, each one resulting from a deficiency of a particular lysosomal protein or, in a few cases, from nonlysosomal proteins, that are involved in lysosomal biogenesis. Most LSDs are inherited in an autosomal recessive manner, with the exception of Fabry disease and mucopolysaccharidosis (MPS) type II, which show X-linked recessive inheritance.

The number of LSDs is steadily increasing as new disorders are characterized biochemically and genetically. A deficiency of cathepsin K has recently been described that results in an LSD called *pyknodysostosis*.[1] In the last 2 years, infantile neuronal ceroid lipofuscinosis (NCL), also known disease, has been shown to result from a deficiency of palmityl protein thioesterase,[2-3] and class infantile NCL has been shown to result from a deficiency of a carboxypeptidase.[4] Many LSDs hav classified into clinical subtypes (such as the Hurler-Scheie definition of MPS type I or the infantil juvenile-, and adult-onset forms of Pompe disease), but it is clear that most LSDs have a broad of clinical severity and age of presentation.

With the advent of molecular biology and the characterization of many of the LSD genes, it is nc recognized that the range of severity may in part be ascribed to different mutations within the s However, genotype-phenotype correlations do not always hold. In Gaucher disease, for example sometimes substantial differences in the clinical manifestation of the disease between siblings ar some instances, one sibling is severely affected while another is virtually free of disease.[5] Other including genetic background and environmental factors, presumably play a role in disease prog

Although each LSD results from mutations in a different gene and consequent deficiency of enzy activity or protein function, all LSDs share a common biochemical characteristic in that the disor in an accumulation of normally degraded substrates within lysosomes. The particular substrates and the site(s) of storage vary, although the substrate type is used to group LSDs into broad cat including MPSs, lipidoses, glycogenoses, and oligosaccharidoses.[6] These categories show many similarities within groups as well as significant similarities between groups. Common features of LSDs include bone abnormalities, organomegaly, central nervous system dysfunction, and coars facies.

There have been a number of reports on the prevalence of particular disorders in select populati note is the level of Gaucher disease and Tay-Sachs disease in the Ashkenazi Jewish population, to be 1 per 855 and 1 per 3900, respectively.[5, 7] Prevalences as high as 1 per 18,500 for aspartylglycosaminuria in the Finnish population[8] and 1 per 24,000 for MPS type III in the Nethe have also been reported. In addition, there have been a number of limited studies on the preval some LSDs in different countries.[10-15] However, in general, these studies have not been comprel and have not covered all LSDs. As the development of therapies for this group of disorders proc the possibilities for neonatal screening are explored, it becomes important to obtain accurate val prevalence of the disorders. These data will be required to accurately assess the cost of these di public health care systems and will be a key factor in the adoption of screening and treatment p for LSDs.

In this article, we present a summary of all LSD diagnoses in Australia for the period 1980 throu

METHODS

Jump to
- Top
- Introductio
- Methods
- Results
- Comment
- Author infc
- References

In this study, patients diagnosed as having an LSD have a reduced level of 1 or more lysosomal proteins, which leads to the storage of substrate within their lysosomes and results in the development of clinical problems and a subsequent reduction in their quality of life.

Retrospective data on the enzymatic diagnosis of LSDs, both from patient referrals and prenatal diagnoses for the period January 1, 1980, through December 31, 1996, were collec the National Referral Laboratory, Department of Chemical Pathology, Women's and Children's H Adelaide, Australia, and from the Division of Chemical Pathology, Royal Brisbane Hospitals, Brist Australia. All diagnoses were performed at these 2 centers and this represents all enzymatic ana performed in Australia during this period. No data were collected on the diagnosis of pyknodysos glycogen-storage disease type II-B, or the various forms of Batten disease, which are currently i diagnosed enzymatically in Australia.

Data on the number of births in Australia were collected from the Australian Bureau of Statistics (Canberra). Data were compiled according to disorder, year of diagnosis, and age of patient (inc prenatal diagnoses), and correlated with Australian birth rates for each year. Instances in which were 2 or more affected siblings were identified.

Incidence rates were calculated by dividing the number of postnatal diagnoses by the number of during the study period. Prevalence rates were calculated by dividing the number of postnatal pl prenatal diagnoses by the number of births during the study period. The total number with pren diagnoses who were not live-born were not included in the denominator because this figure was accurately known and would not have made a significant difference to the prevalence figures. Ca frequency was calculated by dividing the prevalence value by 4 and finding the square root. Carı frequency for X-linked disorders was equal to the prevalence values because the incidence of ca should equal the prevalence of affected births for these disorders.

RESULTS

Jump to
- Top
- Introductio
- Methods
- Results
- Comment
- Author infc
- References

For the period January 1980 through December 1996, there were 470 LSD-affected individuals diagnosed in the Australian population. In addition, there were 75 positive LSD prenatal diagnoses for affected fetuses, yielding a total of 545 diagnoses (Table 1). There was no significant increase in the rate of either clinical diagnoses or prenatal diagnoses during the study period (Figure 1). These diagnoses represent 27 different LSDs, whereas there were 10 LSDs for which there were no diagnoses in Australia during this period (Table 2). The prevalence of these disorc ranged from 1 per 57,000 for Gaucher disease to 1 per 4.2 million for sialidosis. The prevalence as a group was calculated to be 1 per 7700 live births. When prenatal diagnoses were not consic incidence for LSDs was 1 per 9000 live births. Mucopolysaccharidosis, a particularly well-defined of LSDs, had a combined prevalence of 1 per 22,500 and represented 35% of all LSDs. Carrier fi were calculated from the prevalence values and ranged from 1 per 119 for Gaucher disease to 1 for sialidosis (Table 1). Comparison of the number of LSD diagnoses in the different states of Au (Table 3) indicates similar prevalence values in all major population centers. The exceptions, Au: Capital Territory, Northern Territory, and Tasmania, are all low-population areas that use genetic in neighboring states and, as such, would have had some patients recorded in those states.

View this table:
[in this window]
[in a new window]

Table 1. Diagnosis of Lysosomal Storage Disorders in Australia*

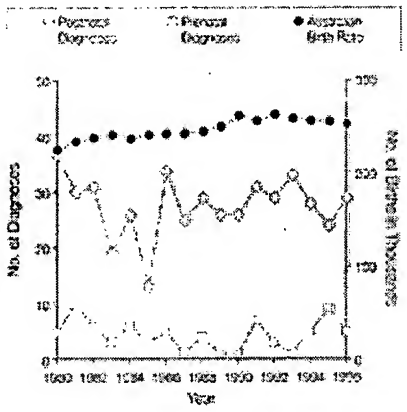


Figure. Postnatal and Prenatal Lysosomal Storage Disord
Diagnoses Made in Australia From 1980 Through 1996

View larger version (9K):
[in this window]
[in a new window]

View this table:
[in this window]
[in a new window]

Table 2. Disorders Not Detected Enzymatically in the Australian Populatio

View this table:
[in this window]
[in a new window]

Table 3. Prevalence of Lysosomal Storage Disorders by State

We determined the median age of the patients at diagnosis for each LSD and report these togetl the low and high values for each disorder (Table 4). Although for some disorders, the number of was not high enough to make these data statistically significant, it still gives an indication of the ages at which these disorders can present. Most disorders (18/27) had more than 10 diagnoses period. These data demonstrate that certain disorders, in particular Fabry disease, can present r late in life, with a mean age at diagnosis of 28.6 years, although for some individuals diagnosis in the first year of life. Clinicians should note the wide range of the clinical spectrum presenting i disorders. In some LSDs, including Krabbe disease, MPS type I, Pompe disease, and Sandhoff di median age at diagnosis was younger than 1 year, although the range of ages in each disorder s reflected a considerable variation in the clinical spectrum.

View this table:
[in this window]
[in a new window]

Table 4. Age at Diagnosis of Patients With Lysosomal Storage Disorders*

Of the 470 clinical diagnoses, there were 79 individuals who had 1 or more affected siblings. The families with twins, 9 families with an index case who in full knowledge had 1 or more additional children, and 26 families who had had 2 affected children before the first had an LSD diagnosed.

these 26 families, the affected individuals were adults (older than 18 years) when the disease w diagnosed.

COMMENT

Jump to
- Top
- Introductio
- Methods
- Results
- Comment
- Author Infc
- References

The prevalence values for individual LSDs clearly define these as rare genetic disorders. Gaucher disease was the most common, with a prevalence of 1 per 57,000 births. However, when taken as a group, LSDs are far more common, with a prevalence of 1 per 7700 births. Each year in Australia there are, on average, 28 LSD diagnoses made, with an additional 4 to 5 prenatal diagnoses. Although exact national figures for the number of MPS referrals are unavailable, in South Australia, 150 to 250 urine screening tests for MPS are performed each year to diagnose MPS, on average patient. White-cell enzymology, which is performed for most other LSDs, is performed on 400 tc patients per year nationally, resulting in an average of 18 diagnoses. These estimates suggest considerable overlap between clinical features of LSDs and other conditions, but may also indica presence of additional as yet undefined LSDs. Although prenatal diagnosis is possible for most L practical prenatal screening tests are available for any LSD.

The life expectancy of a patient with an LSD depends on the particular disorder, the severity, an treatment available. In MPS, this can range from lethal fetal hydrops to an almost-normal life expectancy.[16] In most, if not all, disorders, there is a strong correlation among age at diagnosis and life expectancy. The difference between the median age (2.7 years) and average age (9.7 y diagnosis of an LSD in Australia reflects the relatively few adult patients who have an almost-no expectancy. Based on the average age at diagnosis of 9.7 years and an average of 28 affected i born each year, we estimate that there are currently about 270 individuals with an undiagnosed possibly up to twice that number of patients with a diagnosed LSD in Australia.

That there were only 2 centers involved in the enzymatic diagnosis of LSDs greatly facilitated th collection of data for this study; as a consequence, we have a high level of confidence that few c were missed. Our confidence is supported by the state-by-state breakdown of the prevalence va the LSDs, with the 5 major population centers showing similar prevalence data. Had 1 or more s missed a significant number of cases, this would present as uneven prevalence values among st addition, there was no significant variation in the number of diagnoses made per year during the period; this would suggest that the patient identification rate is constant and close to 100% for t disorders. It is possible, however, that there are some individuals at the less-severe end of the c spectrum of some disorders, particularly in the adult population, in whom an LSD was not diagn Gaucher disease is likely in this group. Similarly, we observed that there was no increase in the prenatal diagnoses for the period of the study; this again reflects the steady rate of diagnosis of disorders.

To calculate incidence, prevalence, and carrier frequency values, we needed to make certain ass We assumed that the rate of postnatal diagnosis was equivalent to the birth rate for each disord postnatal diagnosis was less than the birth rate, as a result of undiagnosed early death, then ou estimates of incidence values would be low. This is unlikely because all unexpected child deaths Australia result in postmortem examinations, including histopathology studies. We also assumed parents of affected individuals were heterozygous for the disorders (with the exception of X-link disorders). If this were not the case, then our estimates of carrier frequency would be low; howe homozygous parents, in what are predominantly childhood disorders, are not common and, as s have little effect on our carrier frequency estimates.

The Australian population is predominantly of British extraction, with a significant contribution fr European countries and, to a lesser extent, Asian countries. As such, this population would be cc with that of most Anglo-Celtic countries. Therefore, these results could be extrapolated to the w Hispanic populations in the United States, Canada, and the United Kingdom. However, a higher p of Ashkenazi Jews in a community may increase the prevalence of Gaucher disease and Tay-Sac disease.

Although no data are available on the ethnic background of those diagnosed as having an LSD, v evidence that the Ashkenazi Jewish population contributed significantly to the figures for either (disease or Tay-Sachs disease in Australia. The Ashkenazi Jewish population in Australia is estima 105,000 and is concentrated in Victoria and New South Wales. Despite this, we see no increase prevalence of either Gaucher disease or Tay-Sachs disease in these states compared with other Australia. This may be the result of outbreeding from the Jewish community into the general po screening program for the detection of Tay-Sachs disease carriers in the Jewish community was commenced in 1994; however, this would have had only a minimal effect on this study, which c period 1980 to 1996.

The cost to the community, in particular the health care system, of individuals with LSDs is signi have calculated the medical costs for a patient with severe MPS I who has not had a bone marro transplant to be approximately Aust $80,000 (US $56,000) per year, based on hospital admissic hospital procedures, and outpatient visits during a 2.5-year period. In addition, such a patient w require full-time nursing home care while attending a special school, further increasing the cost community. Bone marrow transplantation costs, on average, Aust $41,000 (US $29,000). Enzyn replacement therapy for Gaucher disease currently costs between Aust $140,000 and $250,000 $98,000-$175,000) per year, although the cost for enzyme replacement therapy should decreas efficient enzyme production systems are developed. Given that some of the affected individuals less-severe end of the clinical spectrum, the total cost to the community for individuals with an I Australia is thought to be in the tens of millions of dollars per year. Although it is useful to deter cost of these disorders to the community, in particular to the health care system, this represent fraction of the real cost, in human terms, of these disorders.

There were 39 families with more than 1 affected child; this highlights the need for early diagno these disorders because in most cases, there were 2 affected children born before the first was (as having an LSD. Early detection of LSDs, such as that possible in the neonatal screening progr phenylketonuria and other genetic diseases, would provide the option for prenatal diagnosis for more families carrying these disorders. In addition, early detection would maximize the efficacy and proposed therapies for LSDs. The efficacy of these therapies, particularly for those LSDs inv central nervous system and bone pathologies, will rely heavily on the early diagnosis and treatm disorder, before the onset of irreversible disease. A further consideration, critical to bone marrov transplant therapy, is that early diagnosis of the LSD will allow clinicians to take advantage of th of opportunity presented by the naturally suppressed immune system of the neonate to maximi: chances of a successful engraftment. Early intervention has the potential to reduce costs associa LSDs. Studies into the development of neonate screening for LSDs are currently in progress.[17]

Recently, there have been several advances made in the understanding of NCLs, a group of at le disorders classified by age at onset. Previously, these disorders were diagnosed histopathologica than enzymatically and, consequently, we have no incidence data available. However, NCLs are common: estimates of incidence levels range from a global incidence for all forms of 1 per 12,5(per 78,000 for all forms in Germany.[19] In Finland, where there is a particularly high level, incide of 1 per 13,000 for infantile and 1 per 21,000 for juvenile forms have been reported.[20] Clearly, I

going to contribute importantly to the overall prevalence of LSDs. A rate of 1 per 50,000 births f would alter the prevalence of LSDs from 1 per 7700 to 1 per 6700. Further epidemiological studi required for this group of disorders.

AUTHOR INFORMATION

Jump to
- Top
- Introductic
- Methods
- Results
- Comment
- Author inf
- Reference

Funding/Support: This study was supported in part by the South Australian Health Commission and the Women's and Children's Hospital Research Foundation, Adelaide; and the National Health and Medical Research Council of Australia, Canberra.

Acknowledgment: We acknowledge the many referring pediatricians for clinical diagnoses; Sharon Chin, Peter Clements, PhD, Beverley Fong, Vivian Muller, Paul Nelson, Dace F and Greta Richardson for chemical diagnoses performed in Adelaide; Robert Barns, PhD, and Da for chemical diagnoses performed in Brisbane; and Janice Fletcher, MD, and Nicolla Poplawski, M analyses of treatments.

Corresponding Author and Reprints: Peter J. Meikle, PhD, Lysosomal Diseases Research Unit Department of Chemical Pathology, Women's and Children's Hospital, 72 King William Rd, North 5006, South Australia (e-mail: pmeikle@medicine.adelaide.edu.au).

Author Affiliations: Lysosomal Diseases Research Unit and National Referral Laboratory, Depa Chemical Pathology, Women's and Children's Hospital, Adelaide, Australia (Drs Meikle, Hopwood Carey); and the Division of Chemical Pathology, Royal Brisbane Hospitals Campus, Queensland I Pathology Service, Brisbane, Australia (Dr Clague).

REFERENCES

Jump to
- Top
- Introductic
- Methods
- Results
- Comment
- Author inf
- Reference

1. Gelb BD, Shi GP, Chapman HA, Desnick RJ. Pyknodysostosis, a lysosomal disease caused by cathepsin K deficiency. *Science.* 1996;273:1236-1238. ABSTRACT

2. Vesa J, Hellsten E, Verkruyse LA, et al. Mutations in the palmityl protein thioesterase gene causing infantile neuronal ceroid lipofuscinosis. *Nature.* 1995;376:584-587. CrossRef | ISI | MEDLINE

3. Hellsten E, Vesa J, Olkkonen VM, Jalanko A, Peltonen L. Human palmityl protein thioesterase: evidence for lysosomal targeting of the enzyme and disturbed cellular routing in infantile neuronal ceroid lipofuscinosis. *EMBO J.* 1996;15:5240-5245. ABSTRACT

4. Sleat DE, Donnelly RJ, Lackland H, et al. Association of mutations in a lysosomal protein with late-infantile neuronal ceroid lipofuscinosis. *Science.* 1997;277:1802-1805. ABSTRACT/FULL TEXT

5. Beutler E, Grabowski GA. Gaucher disease. In: Scriver CR, Beaudet AC, Sly WS, Valle D, eds. *Metabolic and Molecular Basis of Inherited Disease.* Vol 2. 7th ed. New York, NY: McGraw-Hill Bc 1995:2641-2670.

6. Hopwood JJ, Brooks DA. An introduction to the basic science and biology of the lysosome and diseases. In: Applegarth DA, Dimmick JE, Hall JG, eds. *Organelle Diseases.* New York, NY: Chap Hall; 1997:7-35.

7. Petersen GM, Rotter JI, Cantor RM, et al. The Tay-Sachs disease gene in North American Jew populations: geographic variations and origin. *Am J Hum Genet.* 1983;35:1258-1269. ISI | MEDLI

8. Arvio M, Autio S, Louhiala P. Early clinical symptoms and incidence of aspartylglycosaminuria *Acta Paediatr.* 1993;82:587-589. ISI | MEDLINE

9. van de Kamp JJ, Niermeijer MF, von Figura K, Giesberts MA. Genetic heterogeneity and clinic variability in the Sanfilippo syndrome (types A, B, and C). *Clin Genet.* 1981;20:152-160. ISI | ME

10. Lowry RB, Applegarth DA, Toone JR, MacDonald E, Thunem NY. An update on the frequency mucopolysaccharide syndromes in British Columbia. *Hum Genet.* 1990;85:389-390. ISI | MEDLINE

11. Ozand PT, Gascon G, al Aqeel A, Roberts G, Dhalla M, Subramanyam SB. Prevalence of diffe of lysosomal storage diseases in Saudi Arabia. *J Inherit Metab Dis.* 1990;13:849-861. ISI | MEDLI

12. Krasnopolskaya KD, Mirenburg TV, Aronovich EL, et al. Diagnosis and prevention of lysosom diseases in Russia [published erratum appears in *J Inherit Metab Dis.* 1994;17:111]. *J Inherit M* 1993;16:994-1002. ISI | MEDLINE

13. Czartoryska B, Tylki Szymanska A, Gorska D, Sawnor Korszynska D. Lipidoses detected in P through 1993. *Pediatr Neurol.* 1994;11:295-297. CrossRef | ISI | MEDLINE

14. Michelakakis H, Dimitriou E, Tsagaraki S, Giouroukos S, Schulpis K, Bartsocas CS. Lysosom diseases in Greece. *Genet Couns.* 1995;6:43-47. MEDLINE

15. Coelho JC, Wajner M, Burin MG, Vargas CR. Selective screening of 10,000 high-risk Braziliar for the detection of inborn errors of metabolism. *Eur J Pediatr.* 1997;156:650-654. CrossRef | ISI |

16. Neufeld EF, Muenzer J. The mucopolysaccharidoses. In: Scriver CR, Beaudet AC, Sly WS, Va *The Metabolic and Molecular Basis of Inherited Disease.* Vol 2. 7th ed. New York, NY: McGraw-H Co; 1995:2465-2494.

17. Meikle PJ, Brooks DA, Ravenscroft EM, et al. Diagnosis of lysosomal storage disorders: evalu lysosome-associated membrane protein LAMP-1 as a diagnostic marker. *Clin Chem.* 1997;43:13 ABSTRACT/FULL TEXT

18. Rider JA, Rider DL. Batten disease: past, present, and future. *Am J Med Genet Suppl.* 1988; MEDLINE

19. Claussen M, Heim P, Knispel J, Goebel HH, Kohlschutter A. Incidence of neuronal ceroid-lipo in West Germany: variation of a method for studying autosomal recessive disorders. *Am J Med* (1992;42:536-538. ISI | MEDLINE

20. Santavuori P. Neuronal ceroid-lipofuscinoses in childhood. *Brain Dev.* 1988;10:80-83. ISI | M

RELATED ARTICLES IN *JAMA*

This Week in *JAMA*
JAMA. 1999;281:211.
FULL TEXT

January 20, 1999
JAMA. 1999;281:293-294.
EXTRACT | FULL TEXT

THIS ARTICLE HAS BEEN CITED BY OTHER ARTICLES

Glycosaminoglycan degradation fragments in mucopolysaccharidosis I
Fuller et al.
Glycobiology 2004;14:443-450.
ABSTRACT | FULL TEXT

Results of a Nationwide Screening for Anderson-Fabry Disease among Dialysis Patient
Kotanko et al.
J Am Soc Nephrol 2004;15:1323-1329.
ABSTRACT | FULL TEXT

Brain Pathology 8: 175-193(1998)

Cellular Pathology of Lysosomal Storage Disorders

Steven U. Walkley

Sidney Weisner Laboratory of Genetic Neurological Disease, Department of Neuroscience
Rose F. Kennedy Center for Research in Mental Retardation and Human Development, Albert Einstein College of Medicine, Bronx (NY), USA

Lysosomal storage disorders are rare, inborn errors of metabolism characterized by intralysosomal accumulation of unmetabolized compounds. The brain is commonly a central focus of the disease process and children and animals affected by these disorders often exhibit progressively severe neurological abnormalities. Although most storage diseases result from loss of activity of a single enzyme responsible for a single catabolic step in a single organelle, the lysosome, the overall features of the resulting disease belies this simple beginning. These are enormously complex disorders with metabolic and functional consequences that go far beyond the lysosome and impact both soma-dendritic and axonal domains of neurons in highly neuron type-specific ways. Cellular pathological changes include growth of ectopic dendrites and new synaptic connections and formation of enlargements in axons far distant from the lysosomal defect. Other storage diseases exhibit neuron death, also occurring in a cell-selective manner. The functional links between known molecular genetic and enzyme defects and changes in neuronal integrity remain largely unknown. Future studies on the biology of lysosomal storage diseases affecting the brain can be anticipated to provide insights not only into these pathogenic mechanisms, but also into the role of lysosomes and related organelles in normal neuron function.

Corresponding author:
Steven U. Walkley, DVM, PhD, Sidney Weisner Laboratory of Genetic Neurological Disease, Dept. of Neuroscience, Rose F. Kennedy Center for Research in Mental Retardation and Human Development, Albert Einstein College of Medicine, Bronx, (NY) 10461, USA, Tel.:718/430 4025; Fax:718/430 8821; E-mail: walkley@aecom.yu.edu

Introduction

This year marks a quarter century since the appearance of the landmark publication "Lysosomes and Storage Diseases" (24) in which Hers and Van Hoof and their colleagues presented in full detail their treatise on the then relatively new field of lysosomal disorders. A mere eight years before Hers had proposed, based on his studies of glycogenosis (23), that so-called "storage diseases" were actually caused by deficient activity of lysosomal enzymes (46). According to this view, absence of hydrolytic activity of an individual lysosomal enzyme would result in a catabolic defect and lead to accumulation of a specific metabolic product normally cleaved by that enzyme. At the time most of the known storage diseases were recognized both by their clinical characteristics and by accumulation (hence the term "storage") of a major type of substrate (gangliosides, mucopolysaccharides, etc.). During the period following Hers' proposal the study of storage diseases expanded rapidly and a great number of these diseases were confirmed as being "lysosomal" in origin. These included a variety of enigmatic disorders that had been identified earlier in the century (or before), like Tay-Sachs, Hurler, Niemann-Pick, Krabbe and Gaucher diseases, as well as newer disorders like fucosidosis and mannosidosis. Clearly what had emerged was an explanation for an entire family of disorders believed linked by genetic defects involving individual enzymes in a single organelle, the lysosome. The resulting metabolic blockade and lysosomal storage appeared to be key events responsible for cellular dysfunction and the clinical disease exhibited by the individual. Thus, from gene defect to enzyme deficiency, to intracellular storage and functional compromise of cells, these diseases fulfilled Garrod's classic concept of inborn errors of metabolism (15) as well as any known genetic diseases. There was even optimism for the possible successful treatment of these diseases based on delivery of the missing enzyme to the lysosomal system (10).

Successful delineation of individual storage diseases according to enzyme deficiency and substrate storage has been followed in more recent years by important advances in understanding the molecular genetics of these disorders (42, 63). This explosion of information

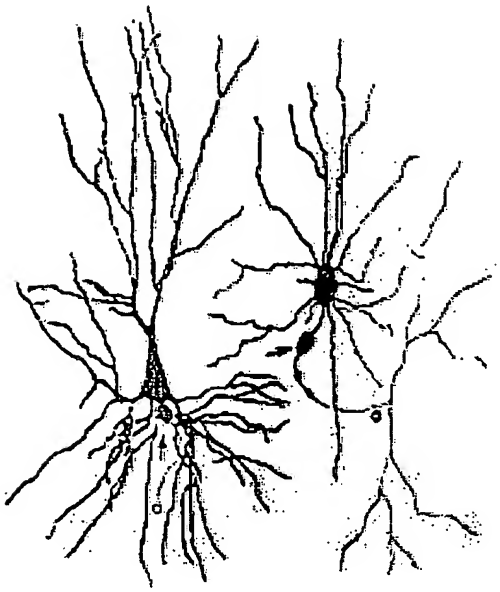


Figure 1. Two of the most conspicuous and well documented examples of morphological alterations that neurons undergo in storage diseases are ectopic dendritogenesis and axonal spheroid formation, yet the link between the primary lysosomal event and these cellular changes is little understood. The illustration above shows two cortical neurons from feline GM2 gangliosidosis. The pyramidal neuron on the left exhibits ectopic dendritogenesis at the axon hillock as it would be seen in a Golgi impregnation (91). The nonpyramidal GABAergic neuron on the right exhibits axonal spheroid formation as would be seen following an intracellular peroxidase injection (33). Immunocytochemical studies have documented the massive abundance of these spheroids within axons of GABAergic neurons (81). Ectopic dendritogenesis does not occur on GABAergic neurons, and axonal spheroids are at best only very rarely seen affecting cortical pyramidal neurons in storage diseases. The conundrum is how does a single lysosomal hydrolase deficiency cause two such diverse events involving both dendrites and axons?

about specific gene defects generated a more complex picture of the overall nature of this family of disorders, compared to the original, simpler view of a single enzyme deficiency being responsible for an individual storage disease. That is, most lysosomal enzyme defects have been found to be caused by multiple gene mutations. For example, over 50 mutations are now identified as affecting the α-subunit of the β-hexosaminidase enzyme, deficiency of which is linked to Tay-Sachs disease and its variants (20). Advances in molecular genetics of storage diseases have also tended to overshadow analysis of events at the other end of the disease spectrum, namely those unanswered questions concerning the important issue of disease process. Specific genetic mutations may be well characterized as causing defective function of individual lysosomal enzymes, but how the resulting metabolic changes then generate the complex events occurring in cells and tissues, particularly brain, remains poorly understood. Neurons are also known to exhibit diverse and often bizarre alterations in morphology secondary to the storage disease process, and may do so in a highly neuron-type specific fashion. For example, some types of neurons in widely diverse kinds of storage diseases exhibit what clearly is one of the most unusual and unprecedented changes of any neurological disease, the growth of new, primary dendrites (Figure 1A). Likewise, other neurons, again in a cell-selective manner, undergo changes in axons, focal swellings or spheroids, at sites far removed from that of the primary lysosomal derangement (Figure 1B). There is every reason to believe that these changes in neuronal morphology have profound consequences for neuron and brain function yet we know little about the link between the lysosomal derangement and the generation of these neuron-specific alterations. In other storage disorders, particularly the Batten family of storage diseases, it is the death of neurons rather than changes in neuronal morphology per se that appears to cause brain dysfunction. This loss of neurons is again a highly cell type specific phenomenon, even though intraneuronal storage is universal, and the reason why particular neurons die is unknown. The purpose of this review is to explore and reexamine some of these unresolved issues involving the pathogenesis of lysosomal diseases, issues of disease process residing at the level of individual organelles and cells, particularly the neuron. It is, after all, the neuron, largely due to its longevity, that provides perhaps the widest array of questions and greatest importance in these disease states. Overall descriptions of the cellular pathology of individual lysosomal storage diseases can be found in excellent recent reviews (e.g., see 35).

The Lysosome and Storage

In his chapter on lysosomes in the above-mentioned text by Hers and Van Hoof (24), Novikoff summarized the then current view of the lysosomal system in normal cells (46). His figure illustrating the lysosomal system (reproduced here as Figure 2) reveals the primary lysosome, filled with a complement of lysosomal hydrolases, as fusing with a variety of other vesicular organelles, including autophagic and digestive vacuoles and multivesicular bodies. These primary lysosomes were believed to have shuttled from the Golgi apparatus

to endocytic vacuoles where fusion occurred, thus forming secondary lysosomes. Residual bodies, viewed as vacuoles containing undigested materials, membrane fragments, myelin figures, etc., were found in normal cells to a degree, but also were recognized as the hallmark of the so-called lysosomal storage diseases. Terry and colleagues carried out the first electron microscopic analysis of storage bodies in Tay-Sachs disease in 1963 and showed the presence of conspicuous multilamellated organelles which they referred to as membranous cytoplasmic bodies or mcb's (70). Similarly, and in the same year, Batten disease neurons were distinguished from those in Tay-Sachs as they contained a radically different, amorphous and "fingerprint" material (98). This discovery finally set Batten disease apart from Tay-Sachs disease and led to the coining of the term "neuronal ceroid lipofuscinosis" to define the former. Within a short time, characteristic storage bodies or "cytosomes" were identified for a host of different types of storage diseases. Indeed, it appeared that major classes of storage diseases could be distinguished by the ultrastructural appearance of their storage material: Glycosphingolipidoses presenting with membranous swirls (mcb's), mucopolysaccharidoses with multilamellar stacks ("zebra bodies"), fucosidosis and mannosidosis with watery or wispy material ("open" inclusions), and so forth (67). The explanation for the characteristic appearances of storage bodies was that cell material entered the lysosome in normal fashion but once there the lack of a specific degradative enzyme led to a buildup of individual metabolic products. Accumulation of this material past a certain critical point was believed to lead to physicochemical interactions with other materials within the lysosome (e.g., cholesterol) and to formation of the characteristic morphological appearance of the residual or storage body for a given disease. Numerous studies even in the early 1960's showed that residual bodies in storage diseases like Tay-Sachs and other neurolipidoses contained acid phosphatase activity suggesting a lysosomal status. Arguments that the storage bodies sometimes lacked histochemical evidence of lysosomal enzymes, or even lysosomal-like delimiting membranes, and thus might not be lysosomal, were eventually interpreted as simply late disease-associated alterations in the lysosome, rather than arguments against the lysosomal disease concept.

Remarkable advances have occurred in our understanding of the lysosome since Novikoff's early summary (29). We now recognize over 40 different enzymes within lysosomes, including glycosidases, lipases, phospholipases, phosphatases, sulfatases, and proteases, and genetic deficiencies and related storage diseases have been identified for most of these (42, 63). We now understand that lysosomal storage in storage diseases may occur secondary to one of several mechanisms: That is, a particular lysosomal enzyme may be absent as a result of a lack of production of mRNA or to unstable mRNA for the enzyme. The mRNA that is present may produce an enzyme of abnormal structure that is less effective in cleaving the appropriate substrate(s). Alternatively, the functional enzyme deficiency may be due to processing or trafficking errors. In these cases, lysosomal enzymes generated in the endoplasmic reticulum or Golgi apparatus may fail to undergo normal post-translational modification leading again to either absent or diminished activity. There may also be abnormal recognition signals leading to errors in trafficking and to misrouting or inappropriate secretion. These latter two groups of processing abnormalities can involve a host of enzymes simultaneously, as is documented in multiple sulfatase deficiency and in I-cell disease. Some lysosomal enzymes are known to require co-factors or protective proteins and their absence has also been linked to defects in lysosomal enzyme function. Lysosomal membrane receptors may themselves be abnormal leading to abnormalities in substrate entry or exit, as is the case in sialic acid storage disease, also known as Salla disease. Finally, several types of storage diseases, most notably Niemann-Pick disease type C and selected forms of Batten disease, stand apart from these well-defined primary lysosomal defects. Current understanding suggests that they result from essentially nonlysosomal events involving trafficking of other types of molecules which secondarily impact the lysosomal system (48, 51).

The relationship between genotype and phenotype at the level of disease process remains poorly defined for most storage disorders. It is known that the specific genetic event precipitating the enzyme deficiency can have profound consequences for disease progression and clinical presentation. These include both the degree of storage and the brain regions most severely affected. Generally speaking, the relative activity of an affected lysosomal enzyme in a given storage disease appears to correlate with the age of onset and severity of disease (9), with most storage diseases appearing as infantile, late infantile, juvenile, or adult-onset variants (63). The existence of compound heterozygotes in which patients possess two dissimilar mutant alleles has been documented to lead to highly unusual disease variants as seen, for example, with GM2 gangliosidosis (20).

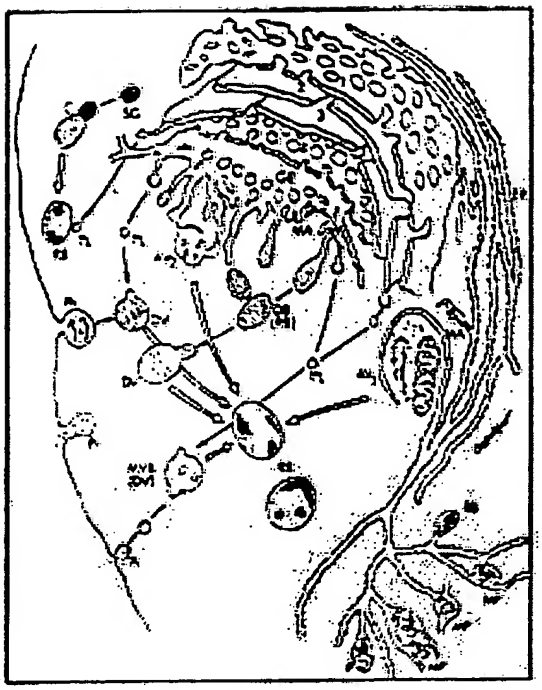


Figure 2. An illustration from Novikoff (46) showing the relationship between lysosomes and other organelles at the time of the development of the lysosomal storage disease concept. (1°L, primary lysosome; AV, autophagic vacuoles; C, crinophagy; DB, dense body; DV, digestive vacuole; ER, endoplasmic reticulum; GE, Golgi; MA, microautophagy; MP, microperoxisome; MVB, multivesicular body; P, peroxisome; Ph, phagocytic vacuole; Pi, pinocytic vacuole; RB, residual body; SG, secretory granule.) (From 46, with permission)

Storage disease-related effects also have been identified in phenotypically normal heterozygotes, as has been reported in a feline model of Niemann-Pick disease type C (7). In this case, occasional cortical neurons exhibited ganglioside storage in a manner very similar to diseased cells in homozygous affected individuals. Non-CNS abnormalities were also detected.

The Endosomal-Lysosomal System. Materials are known to be delivered to lysosomes by multiple pathways, including those associated with endocytosis, autophagy, and phagocytosis. One of the best studied of these pathways relative to the lysosome is the endosome. Indeed, an important advance in the modern understanding of the lysosome as an organelle relates to its important functional association with endosomes and the concept of the endosomal-lysosomal system (1, 34, 43). This association is based in part on the finding that lysosomal enzymes are localized to endosomal compartments, most notably late endosomes, and that the pH of these organelles is acidified (pH 5-6), though not as much so as lysosomes themselves (pH 4-5). There are currently two dominant hypotheses that attempt to explain the relationship between endosomes and lysosomes. The "maturation" model evolved from earlier views of the lysosomal system as summarized in Novikoff's diagram (Figure 2). That is, secondary lysosomes (shown in the figure as digestive vacuoles) are formed following fusion of primary lysosomes containing newly synthesized lysosomal enzymes from the Golgi apparatus, and endosomes (referred to as phagocytic or pinocytic vacuoles in this same diagram). Thus, according to this view, the early endosome gradually matures into a late endosome and, following fusion with primary lysosomes, its functional status is gradually converted to that of a lysosome. A second hypothesis, the so-called "stable compartment" model, argues that early and late endosomes are separate stable populations of organelles. Significant differences in pH, in the presence of specific rab proteins, and in mannose-6-phosphate receptors and lysosomal hydrolases are supportive of the latter model. These models have been widely discussed and compared (3, 21, 40, 49).

A more complete understanding of the endosomal-lysosomal system likely will provide important insights into many of the major unanswered questions involving pathogenesis of certain types of lysosomal storage diseases. That is, viewing storage diseases as wholly lysosomal phenomena and the lysosome as a mere end-organelle for catabolic processing provides little insight into pathogenic mechanisms. If catabolic enzyme defects lead simply to lysosomal storage and no "extra-lysosomal" consequences, one must almost necessarily predict an outcome of simple cell death secondary to cell swelling to account for the neurological dysfunction that characterizes these diseases. Indeed, simple "cytotoxicity" following mechanical disruption of cells has historically, and often, been invoked as a sort of shorthand explanation for cell dysfunction in lysosomal diseases. But the cell-selective consequences of the storage disease process mentioned earlier (and as described in detail below) argue strongly that significant extra-lysosomal events are set in motion by deficiencies of single lysosomal enzymes. How might these occur? At least four possible mechanisms that reach beyond the mere mechanical disruption hypothesis can be cited: (i) Material not catabolized within the lysosome may escape this organelle and in turn have deleterious effects elsewhere in the cell. These effects could be directly toxic or they could impact other metabolic pathways and

alter cell function. (ii) The massive accumulation of unprocessed compounds within the lysosome (e.g., gangliosides) may deprive the cell of certain precursor molecules, leading to a compensatory upregulation or dysfunction of other metabolic pathways or organelles (e.g., see 62). (iii) Abnormalities of the lysosomal system may lead to a reduced entry of materials into lysosomes and to subsequent increases of this compound elsewhere in the cell, with detrimental consequences. (iv) Defective lysosomal enzyme activity may adversely effect the normal functioning of other related organelles, most notably endosomes.

Few of the above possibilities have been rigorously tested for individual types of storage diseases. Perhaps the most intriguing of these is the latter one based on the close association between lysosomes and the endosomal system, and the potential role of lysosomal enzymes within endosomes. If, in storage diseases, both the endosomal and lysosomal compartments are actively involved in the "storage" process, disruption of a variety of signal transduction and other events could be envisaged that could account for the dramatic changes induced in neurons secondary to the enzyme defect. Whereas the lysosome is still most often regarded as a site of end-stage processing for macromolecules, the endosome is viewed as a much more dynamic player in cellular events. Endosomes have been documented as being central to the internalization and processing of cell surface receptors and to playing a role in transmembrane signaling (3). However, not only are many receptors known to be internalized via endosomes, but gangliosides and other cell surface constituents destined for lysosomal degradation are also believed to follow similar trafficking (60). Indeed, some types of membrane receptors that cycle by way of endosomes have been implicated as having functional relationships with specific types of gangliosides. For example, EGF receptors, reportedly associated with GM3 ganglioside (5), accumulate in coated pits after binding EGF and subsequently appear in early and late endosomes, and eventually are degraded in lysosomes. However, evidence is emerging that the internalization process is not simply a mechanism of deactivation of this receptor. Instead, there appears to be both continuation and augmentation of EGF receptor activity within endosomes, including receptor tyrosine phosphorylation which exceeds that attained by the plasmalemmal EGF receptor population (74). Numerous studies have linked modulation of EGF receptor function with GM3 ganglioside, whereby GM3 appears to act to inhibit the formation of EGF receptor dimers after stimulation of cells with EGF (5). Further,

Disease/Neuron	With Ectopic Dendritogenesis	Without Ectopic Dendritogenesis
Types of *Neuronal Storage Diseases:*	- GM1 Gangliosidosis - GM2 Gangliosidosis - Niemann-Pick disease type A - Niemann-Pick disease type C - Mucopolysaccharidosis type I - a-Mannosidosis	Batten disease Fucosidosis
Types of *Neurons:*	- Cortical Pyramidal Neurons - Multipolar Neurons of the Amygdala and Claustrum - Granule Cells of the Fascia Dentata - Spiny Neurons of the Striatum	- Cortical Nonpyramidal Neurons - Motoneurons of the Brain Stem and Spinal Cord - Principal Relay Neurons of Thalamus - Purkinje Cells and other Cerebellar Neurons

Table 1. Examples of neurons and neuronal storage diseases with and without ectopic dendritogenesis. The same types of neurons in each disease are vulnerable to dendritic sprouting.

gangliosides and other glycosphingolipids have shown positive or negative modulatory effects not only on EGF receptors, but also on receptors for NGF, FGF, PDGF, p60c-src and insulin (5, 8, 13, 41, 53, 95). Interestingly, ligands for all of these receptors, as well as cholera toxin (a ligand for GM1 ganglioside), have been implicated in endosomal signal transduction pathways (3).

Effects on Neurons

It has long been appreciated that neurons in storage diseases not only exhibit somatic swelling secondary to the abnormal accumulation of material, but also reveal a variety of other somatic, dendritic and axonal changes. In the earliest papers by Sachs and colleagues on cases of what was then referred to as amaurotic family idiocy (now called Tay-Sachs disease), neurons were described as being swollen and filled with abnormal material (55, 56). Particular note was made of enlargements within the basilar dendrites of diseased cortical neurons and contrast was drawn to spinal motoneurons which exhibited swelling but no dendritic changes. Thus an appreciation of the concept of neuron type-specific changes came early to the study of neuronal storage diseases. In later years so-called axonal swellings were also noted in this and other storage diseases, and terms like "axonal torpedoes", "torpedo bodies" and "axonal spheroids" were variously applied. Use of these often ill-defined terms, coupled with basic misunderstandings as to specific structures actually involved, led to erroneous notions about the cellular pathology of many lysosomal storage diseases.

The difficulty inherent in an analysis of the cellular pathology of storage diseases is aptly demonstrated in a

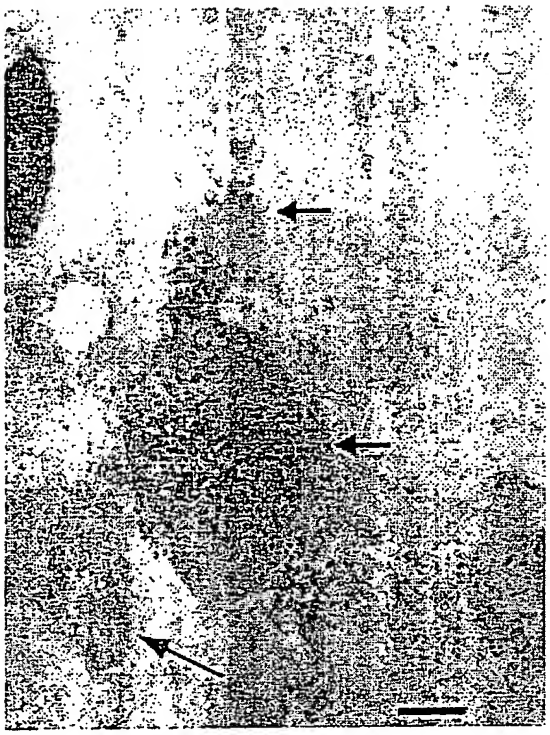


Figure 3. PAS stain of a 2 μm thick plastic section of enlarged neurons in the cerebral cortex from a cat with GM1 gangliosidosis. The massive lysosomal system storage can be seen as PAS-positive perikarya and meganeurites (long arrow). Smaller spherical structures (short arrows) are PAS negative and are axonal spheroids. Calibration bar equals 15 μm.

simple PAS stain of cerebral cortex in a case of ganglioside storage disease (Figure 3). It is readily evident that the neuron at the center of the field is filled with an abnormal accumulation of PAS-reactive material and that the nucleus is displaced into the base of the apical dendrite. Another PAS-positive torpedo-shaped structure is nearby which lacks a nucleus. Structures similar to the latter were observed by Sachs and Strauss (57) in their early studies of Tay-Sachs disease and they are now known to represent parasomatic swellings located at the axon hillock of neurons, as will be described below. Also seen in Figure 3 are two barely visible spherical structures that do not contain PAS-reactive material, one sitting immediately adjacent to the perikaryon, the other in the nearby neuropil. We now recognize these structures to be axonal in nature, so-called axonal spheroids, and to be distinctly different from the other cellular enlargements.

There was considerable appreciation for the existence of unusual swellings on Tay-Sachs disease-affected pyramidal neurons in many early studies (most notably see Bielschowsky, ref. 4). However, an accurate definition of the true nature of these changes had to await the application of modern principles of neurobiology coupled with, ironically, a time-honored staining method dating to the days when storage diseases were first discovered, the Golgi technique (52). Beginning in 1976, Purpura and colleagues demonstrated unequivocally that the parasomatic enlargements of Tay-Sachs disease were not enlargements of basilar dendrites but were of axon hillock origin (see Figure 4). The finding was of crucial importance because it indicated that this enlargement consisted of "new" dendritic-like membrane rather than simply being an enlargement of an existing (basilar) dendrite. This discovery also clearly distinguished dendritic changes from axonal changes and argued against the use of ill-defined terms, like torpedo bodies, to characterize such structural abnormalites. Thus two distinct types of cellular alterations emerged as characteristic of many storage diseases, namely meganeurites and axonal spheroids.

Meganeurite formation in storage diseases. According to the early studies of Purpura and colleagues, meganeurites were parasomatic enlargements within the axon hillock and thus were proximal to the initial segment of the axon (52, 85). They appeared to occur secondary to storage, i.e. as part of a volume expansion by the neuron, since they always contained massive numbers of storage cytosomes identical to those found in the rest of the perikaryon. They were found only on certain types of neurons while other types, just as Sachs had reported, appeared to undergo simple somatic enlargement secondary to storage. Although originally described as being composed of dendritic-like membrane in diseases like Tay-Sachs, subsequent studies of other storage diseases revealed that there were really two classes of meganeurites (75, 76). Some were "spiny" and covered with dendritic-like spines, neuritic extensions and synapses and therefore appeared to be composed of dendritic-like membrane. Others were "aspiny" and lacked any evidence of dendritic spines or new synapse formation. Detailed studies of animal models of ganglioside storage diseases further revealed that both spiny and aspiny meganeurites could occur on specific populations of neurons, whereas in other diseases, like Batten disease (84, 93), only aspiny meganeurites were found (Figure 5). The issue was further complicated by the presence of some neurons exhibiting growth of ectopic dendritic membrane in the form of synapse-covered neuritic sprouts in the absence of a meganeurite. An

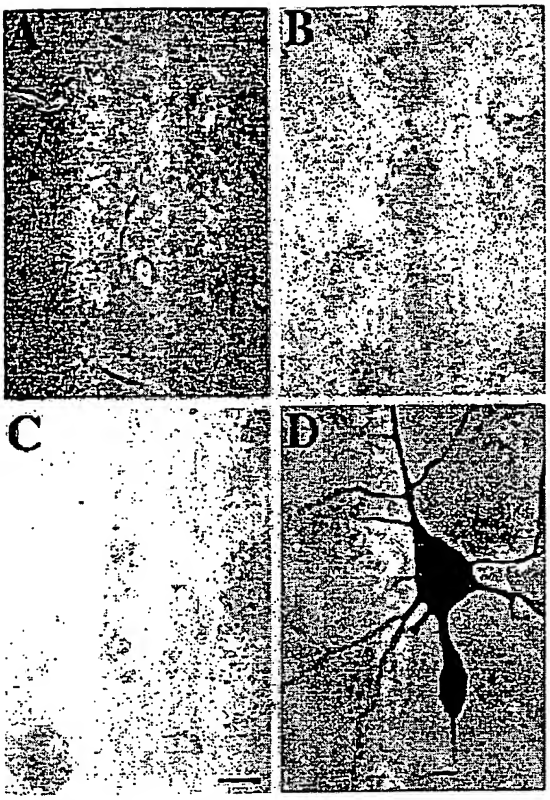


Figure 4. Studies using a histochemical (ferric ion - ferrocyanide staining) technique to identify the axonal initial segment have shown that meganeurites occur proximal to this region and spheroids distal to this region (85). A. normal neurons with dark staining at the initial axonal segment; B. layer III pyramidal neuron with a meganeurite with initial segment staining distal to this structure; C. nonpyramidal-like neuron with an axonal spheroid and initial segment staining between the perkaryon and the spheroid. D. layer III neuron with a meganeurite as visualized with a Golgi stain. (A-C, 2 μm plastic sections counterstained with safranin red. D, Golgi stain). Calibration bar equals 18 μm.

example of this type of neuron is shown in Figure 1A. Such ectopic neurite-bearing neurons, however, were commonly associated with other neurons of the same type that demonstrated the presence of spiny meganeurites. Thus what emerged was the view that specific types of neurons in certain storage diseases appeared capable of undergoing elaboration of new dendritic membrane at the axon hillock, with this new membrane occurring either as neuritic sprouts or as spiny meganeurites (or both). Other storage disorders, like Batten disease, had pyramidal and other neurons with aspiny meganeurites only. In these cases, the meganeurite was viewed exclusively as a volume-accommodation event

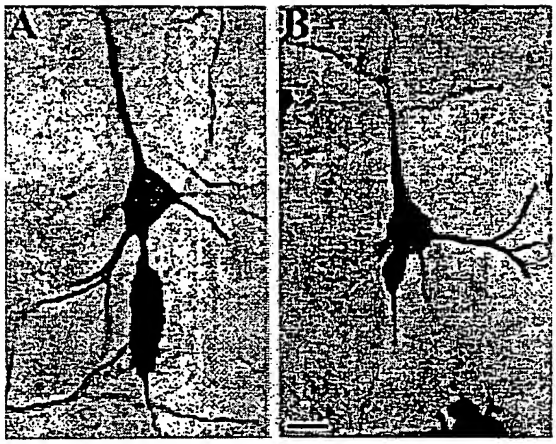


Figure 5. Comparison of pyramidal neurons with a "spiny" meganeurite as seen in feline GM1 gangliosidosis (A) and an "aspiny" meganeurite as seen in canine Batten disease (B). The former represents new, synapse-covered dendritic-like membrane, whereas the latter is simply a volume increase in the cell, as described in the text. Golgi stains. Calibration bar equals 15 μm.

since there was no evidence of new dendritic membrane or new synapses being present. As shown in Figures 6-7 and summarized in Table 1, a variety of types of neurons and storage diseases have been shown to exhibit ectopic dendritogenesis, whereas other cell types and diseases lack this change. An important finding in these studies was that the same types of neurons in all diseases characterized by ectopic dendritogenesis consistently demonstrated the capacity to undergo new dendrite growth. That is, only a few select types of neurons appeared capable of undergoing this process, and these were always the same, even in metabolically diverse storage disorders (75, 76, 78).

Ectopic dendritogenesis and neuronal storage. The discovery that pyramidal neurons in Tay-Sachs disease undergo regrowth of new dendritic membrane in the form of spiny meganeurites and secondary neuritic processes led Purpura and Suzuki to propose two key hypotheses: Firstly, they argued that these structures and their related aberrant synaptic inputs occurring proximal to the axonal initial segment could be responsible for some aspects of brain dysfunction in these diseases. Secondly, they proposed that gangliosides (the primary storage material in this disease) might in some way be linked to the regrowth of new dendritic membrane. It was subsequently discovered that ectopic dendritogenesis occurred in both ganglioside and non-ganglioside storage diseases and the same types of neurons were

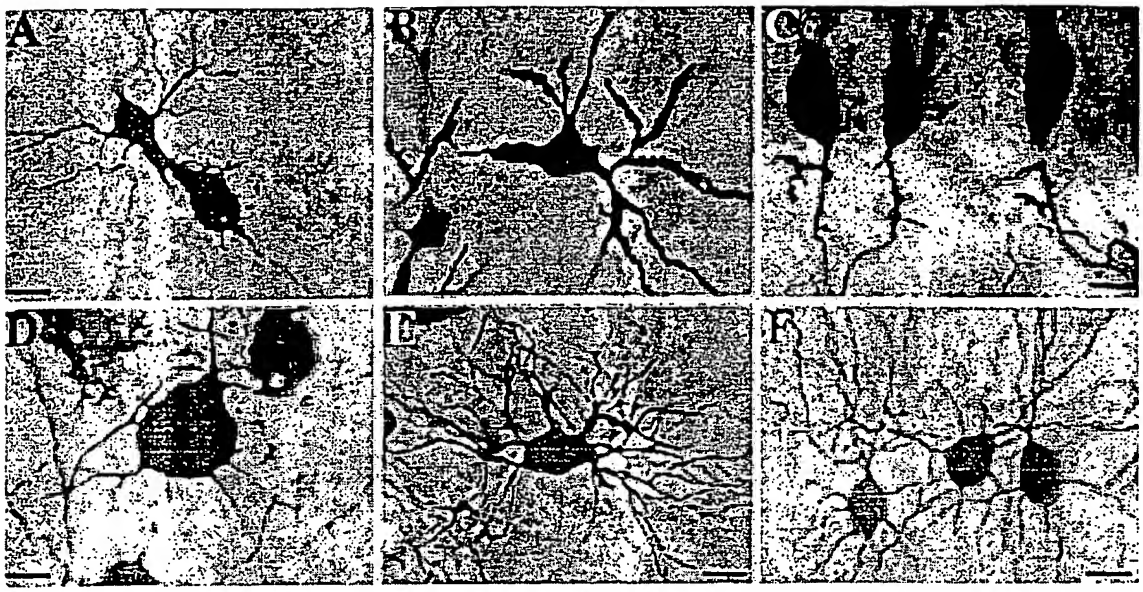


Figure 6. Golgi impregnations of neurons in feline GM1 gangliosidosis showing types of cells susceptible to new dendrite growth. Spiny meganeurites were observed on multipolar cells of the claustrum (A) and caudate nucleus (B). Ectopic dendritic membrane was observed sprouting from the axon hillock region of occasional granule cells of the fascia dentata (cells that normally lack basilar dendrites) (C). Cells that lacked meganeurites or ectopic dendrites included motoneurons (D, oculomotor nucleus), principle neurons of thalamic relay nuclei (E, nucleus ventralis basalis) and nonpyramidal neurons of cerebral cortex (F). Golgi stains. Calibration bars equal A (also for B) 30 μm; C, 15 μm; D, 30 μm; E, 25 μm; F, 20 μm.

consistently affected (79, 80, 82, 83, 88, 91) (Figures 6-7) (Table 1). It could be argued that the presence of ectopic dendritogenesis on the same types of neurons across diverse types of storage diseases indicates the importance of some intrinsic feature unique to these particular neurons. The de novo sprouting of new primary dendrites is a highly unusual event and has not been reported to occur under other circumstances. An obvious question therefore was whether the neurons with ectopic dendrites had specific characteristics in common. Although all such neurons possessed dendritic spines, other dendritic spine-possessing neurons (e.g., Purkinje cells) did not sprout new dendrites. Likewise, axon projection status, neurotransmitter-type, and other features also were not unique to neurons with ectopic dendrites. It is true that the three classes of cells most conspicuously involved with ectopic dendritogenesis, cortical pyramidal neurons and multipolar cells of the amygdala and claustrum, are telencephalic-derived neurons of similar pyramidal-like morphology, but no other common features are evident in terms of cell type.

A consideration of the wide spectrum of storage diseases characterized by ectopic dendritogenesis likewise reveals no common metabolic link (Table 1). And since some types of storage diseases lacked the phenomenon, lysosomal compromise itself appeared an unlikely explanation. Closer examination of one particular kind of storage disease, α-mannosidosis, did, however, provide a crucial clue. This disease was found to exhibit ectopic dendritogenesis in a very limited fashion with only 15-20% of pyramidal cells being affected (although all neurons showed storage) (18, 88). In analyzing neurite-bearing cortical pyramidal neurons in this disease it was readily discovered that these cells consistently contained membranous storage material (Figure 8). This was in clear contrast to adjacent pyramidal neurons lacking ectopic dendrites as they contained only typical wispy or clear inclusions. This remarkable contrast in storage material between neurons was also found to be readily apparent in PAS and toluidine blue-stained 2 μm plastic sections of cerebral cortex. This finding not only illustrated the unique vulnerability of individual neurons to the secondary consequences of the primary metabolic defect, but also suggested that glyco-lipid storage was a feature of neurite-bearing pyramidal neurons in α-mannosidosis. Viewing this discovery within the wider context of all other types of storage diseases exhibiting ectopic dendritogenesis suggested that the common metabolic change underlying the sprouting might be storage of a particular glycolipid, most likely a ganglio-

side. Through a series of studies this fact was indeed established, with GM2 ganglioside shown to be the one metabolic product consistently elevated in the cerebral cortex of all storage diseases characterized by ectopic dendritogenesis (64, 87). By immunocytochemistry, GM2 ganglioside was further localized to vesicular structures in the cytoplasm of ectopic dendrite-bearing pyramidal neurons (77) (Figure 9). Whether all such vesicular structures are actually tertiary lysosomes is not yet established, nor is it yet known why elevations in GM2 ganglioside occur in pyramidal neurons in so many types of storage diseases (apart from GM2 gangliosidosis). However, it is known that the elevations in GM2 ganglioside precede the appearance of ectopic dendritic sprouting (18) and that ectopic dendritogenesis is most common in GM2 gangliosidosis (64). These findings indicate that the increases in GM2 are likely a cause, and not a consequence, of ectopic dendritogenesis (87).

It has also recently been shown that normal developing cortical neurons undergoing dendritogenesis also express GM2 ganglioside in a punctate, vesicular pattern, with the GM2-immunoreactivity disappearing as dendritic maturation progresses (19). The identity of these vesicular structures remains to be determined, but their placement near the Golgi apparatus and in nearby cytoplasm is consistent with current views on ganglioside synthesis and trafficking: Gangliosides are synthesized by a series of glycosyltransferases located in the Golgi apparatus, and after synthesis, they are believed to be translocated to the plasmalemma by vesicular membrane flow (58, 59, 61, 62, 72, 73, 96). Evidence suggests that some types of gangliosides may undergo axonal transport (e.g., see 25), whereas others are transported to the soma-dendritic domain. Exocytic transport vacuoles budding from the Golgi apparatus are believed to move to the plasmalemma where membrane fusion results in ganglioside being inserted with their hydrophilic head groups on the surface of the neuron. Here, current understanding is that gangliosides have the potential to move in the plane of the membrane and to interact with a variety of membrane receptors and other proteins on which they may have modulatory effects (22, 26, 36, 62, 95, 97). Endocytosis and subsequent recycling of gangliosides may involve internalization via coated pits, followed by endosomal transport to lysosomes (61, 62).

The simplest explanation for why GM2 ganglioside is elevated in storage diseases other than GM2 gangliosidosis is that it is due to a generalized lysosomal derangement that impacts function of its catabolic

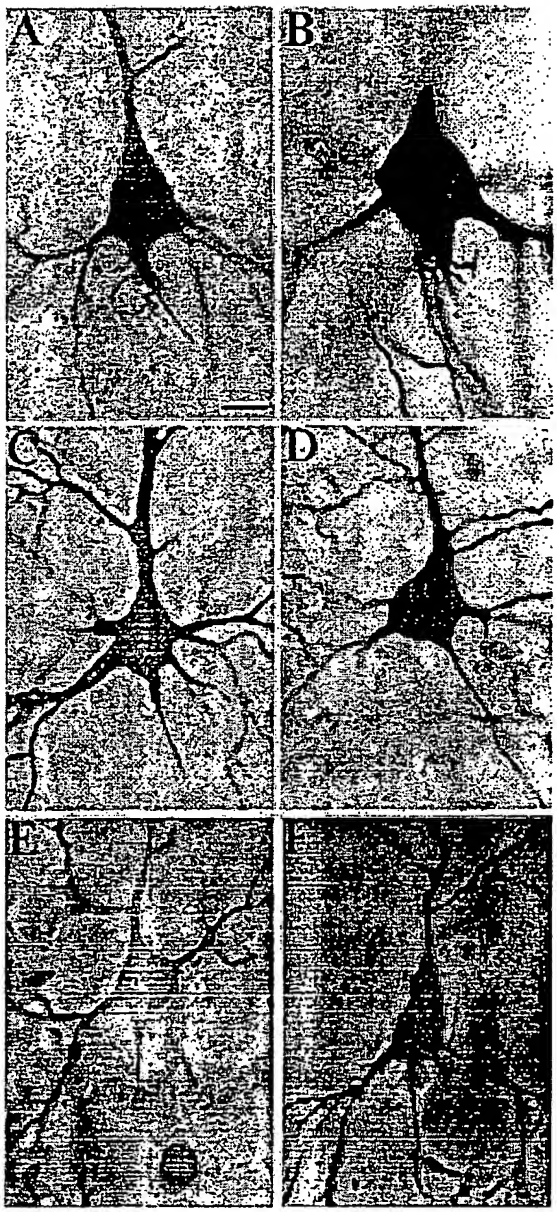


Figure 7. Examples of ectopic dendrite growth on cortical pyramidal neurons in a variety of lysosomal storage disorders in feline models. A. GM1 gangliosidosis; B. swainsonine-induced α-mannosidosis; C. mucopolysaccharidosis type I; D. Niemann-Pick disease type A; E. GM2 gangliosidosis; F. Niemann-Pick disease type C. Golgi stains. Calibration bar equals 18 μm.

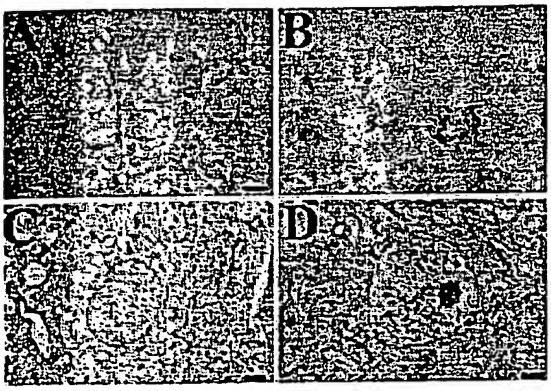


Figure 8. Studies examining the nature of the storage material in cortical neurons in feline α-mannosidosis revealed adjacent neurons with radically different types of storage. (A) toluidine blue (TB) stained 2 μm plastic section showing diversity of staining of storage material. Neuron on the far right exhibits TB-positive storage, the others are negative. (B) Similarly, PAS staining reveals occasional positive cells (on right) whereas most cells exhibit storage, but no staining. C and D, electron micrographs of neurons in the cerebral cortex showing typical clear, open inclusion apparent in most cells (C) as compared to those less frequent neurons containing cytosomes with membranous storage material (D). Calibration bars equal 10 μm for A and B; C, 4 μm; D, 3 μm.

enzyme, β-hexosaminidase. Yet there is no evidence suggesting a consistent and specific defect in the activity of this enzyme in these disparate storage diseases. Similarly, it could be conjectured that changes in ganglioside synthesis have occurred, but this issue has not been systematically studied in storage diseases. In normal developing neurons there is no reason to assume that the GM2-immunoreactive organelles are exclusively lysosomes. Rather, based on current knowledge of ganglioside synthesis and trafficking, exocytic vacuoles and endosomes are the likely organelles involved. Therefore it is not clear how the expression of an individual ganglioside in such diverse conditions, mature neurons undergoing lysosomal storage and normal, immature neurons undergoing dendritic differentiation, could be linked to regulation of dendritic sprouting. Clearly the suggestion is that there may be a common location of GM2 ganglioside in both circumstances. In normal neurons, as mentioned earlier, current hypotheses suggest that gangliosides function by modulating activity of growth factor or other receptors located in the plasmalemma. Both receptors and gangliosides are believed to enter the cell via endosomal trafficking prior to lysosomal degradation. Thus, the plasmalemmal-endosomal interface appears a likely common site where GM2 may function to influence receptors, which

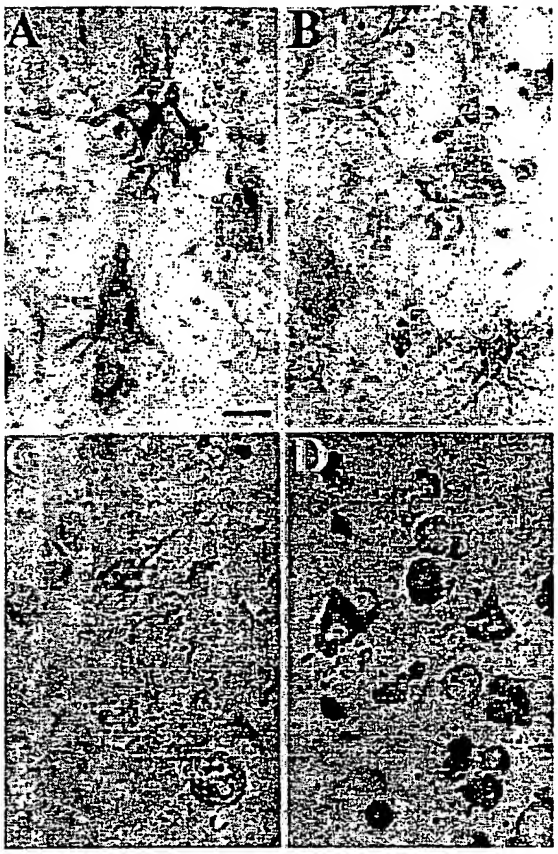


Figure 9. Immunocytochemical studies to localize GM2 ganglioside in storage diseases reveals that it is present in cortical pyramidal cells when these neurons exhibit ectopic dendritogenesis based on Golgi staining (A. α-mannosidosis; C. mucopolysaccharidosis type I; D. Niemann-Pick disease type C). Disease lacking ectopic dendrite growth on the pyramidal cell population also lack evidence of GM2-immunoreactivity in these cells although GM2 elevations may be seen in glial cells (B. fucosidosis). PAP immunocytochemistry with TB counterstain; Calibration bar equals 20 μm.

in turn through interactions with second messengers, could lead to dendritic outgrowth. There are also other indications that the plasmalemma in ganglioside storage disease neurons is not normal (90). Fine gold labelling using a Golgi-EM method has revealed numerous membrane redundancies (Figure 10) resembling "ruffles" and other cell surface phenomena believed associated in other cell types with macropinocytosis, a form of endocytosis (69).

The above findings suggest that during active dendritogenesis GM2 ganglioside may be associated with the endosomal system in both storage disease-affected and

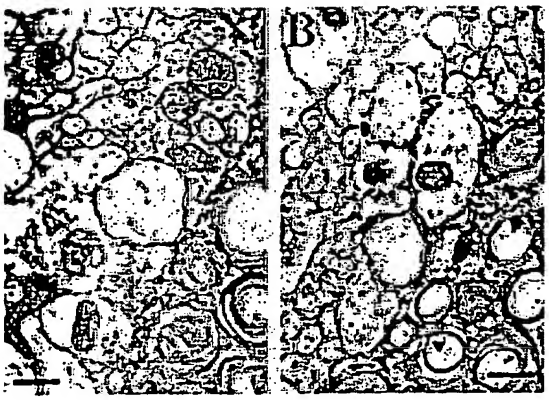


Figure 10. Combined Golgi-EM analysis of cortical neurons in a feline model of ganglioside storage disease reveals the presence of membrane "ruffles" on the surface of the neuron. Gold particles replace the silver chromate of the Golgi reaction and label thin plasmalemmal extensions. Calibration bars equal for A, 0.3 μm; B, 0.4 μm.

normal developing neurons. This is of particular importance since endosomes, as discussed earlier, have been documented as playing a role in the internalization and processing of cell surface receptors as well as in transmembrane signaling. These studies offer the provocative suggestion that gangliosides may exercise their modulatory influence over cell signaling specifically at the plasmalemmal-endosomal interface. Understanding the precise localization of GM2 ganglioside during dendritic sprouting in storage diseases is clearly of central importance in unraveling its role in this phenomenon.

Axonal spheroid formation in storage diseases. Just as meganeurites are structural enlargements proximal to the initial segment of the axon, axonal spheroids are enlargements distal to this region (Figure 4). There is also a second critical distinction: Whereas meganeurites contain storage material consistent with the specific defective lysosomal hydrolase, axonal spheroids contain a distinctly different array of materials with ultrastructural features that are the same across many types of storage disorders. That is, they consist of tubulovesicular profiles, dense bodies, mitochondria, and related materials. Most importantly, they do not contain cytosomes equivalent to those observed in the perikaryon or meganeurite for a given type of storage disease.

For many years axonal spheroids were presumed to be "nonspecific" phenomena potentially occurring in all types of neurons. However, in studies of animal models of storage diseases using immunocytochemical methods it has been determined that the vast majority of the

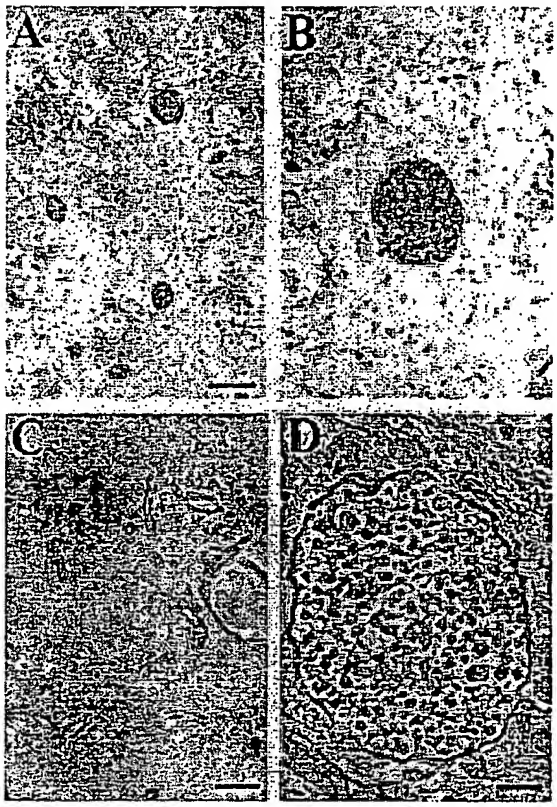


Figure 11. Studies of axonal spheroids in the cerebral cortex of feline models of lysosomal diseases reveal that they predominate in GABAergic neurons. Immunocytochemical staining for GAD, the synthetic enzyme for GABA, identifies spheroids within axons of this type of neuron (A, B). In routine plastic sections without immunostaining spheroids are more difficult to identify (C). EM analysis of spheroids (D) reveal a typical ultrastructure that is similar across brain regions and types of storage diseases. Calibration bars equal A, 20 μm; C (and B) 5 μm; D, 1.2 μm.

spheroids can be labeled with antibodies specific for GABAergic cell populations, namely GAD (glutamic acid decarboxylase) and calcium binding proteins (parvalbumin, calretinin, calbindin) (38, 81). Indeed, this immunocytochemical approach to identifying spheroids has also shown that they are far more abundant in gray matter areas, like cerebral cortex, than previously appreciated (Figure 11). Antibodies to specific cytoskeletal elements like neurofilaments or microtubule associated proteins only rarely label spheroids (Figure 13). This finding is consistent with ultrastructural evidence showing a lack of accumulation of such cytoskeletal elements within spheroids. Immunocytochemical and other studies, including direct neuronal injection studies (Figure

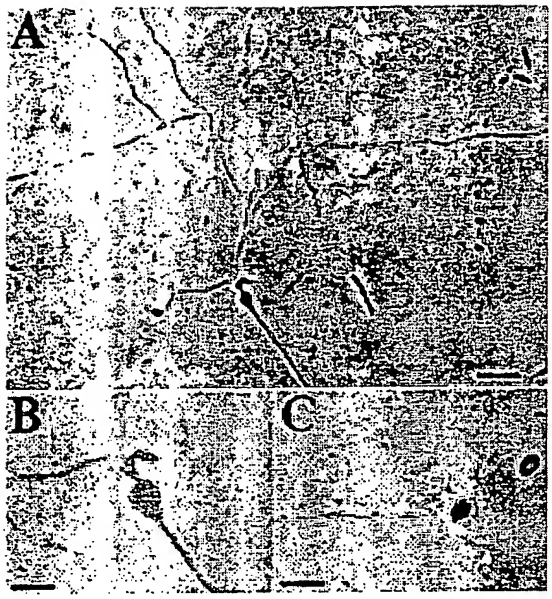


Figure 12. Studies in which GABAergic cells were directly injected with horseradish peroxidase show morphology of axons and spheroids in detail. **A.** Axonal field and partial view of cell body of a putative GABAergic neuron (based on somadendritic morphology); **B.** Spheroid in mainstem dendrite appears flatter on proximal side suggestive of retrograde filling; **C.** Smaller spheroids were also visible in the axonal terminal fields of this cell. Calibration bars **A**, 40 μm; **B**, 20 μm; **C**, 15 μm.

12) (33), have also shown that spheroids occur in axons at a variety of sites. A given axon may have numerous spheroids. These may occur proximally and near the cell body (but distal to the axonal initial segment), at various sites along the length of an individual axon, or in the terminal axonal field (including within synapses).

Axonal spheroids in storage diseases do not appear to be "retraction bulbs" of dying axons (Figures 12-13). Rather, they are swellings of significant size that occur along the length of an axon with axonal continuities clearly visible on both proximal and distal sides. These structural characteristics of spheroids suggest that they may be caused by defects in axoplasmic transport. Accumulations of similar heterogeneous organelles have been reported to occur distal to crush or low temperature lesions in axons, indicating that these types of materials are characteristic of a block in retrograde transport (50, 66, 71). The close similarity of accumulated material in the lesion studies to that of spheroids in storage diseases suggests that the latter may also be secondary to a block in retrograde transport. One type of neuron most susceptible to spheroid formation, the cerebellar Purkinje cell, also is a cell type that is highly vulnerable to cell death in storage diseases. In this case cell death follows spheroid formation and may be secondary to it. That is, spheroid formation and a block in retrograde movement of a growth factor or other element essential to the survival of this class of neuron might be responsible for their selective death. Presently there is less evidence in support of a defect in anterograde transport to explain the formation of spheroids. For example, in GABAergic neurons, the anterogradely-transported enzyme, glutamic acid decarboxylase (GAD), fills the spheroid (hence allowing their identification, see 81), but GAD-immunoreactivity in synaptic terminals of these cells appears to be normal.

In attempting to address the question as to why a defect in axoplasmic transport might occur in a wide variety of primary lysosomal diseases, one logical possibility is that the lysosomal system compromise in perikarya simply deprives the axon of a key component required for the normal movement of organelles or other material. This could involve, for example, either deficiencies of lysosomes or lysosomal hydrolases, the ability to acidify lysosomes, or key components of molecular motor transport mechanisms. As for lysosomes, their possible occurrence in axons has received remarkably little direct examination. Retrogradely-carried endosomes, e.g. from synaptic terminals, are generally believed to fuse with lysosomes only after endosomes have reached the neuronal cell body (for discussion see refs. 28, 43). However, some older (and recent) research findings do provide substantial support for the presence of at least small numbers of lysosomes and lysosomal enzymes in axons (6, 16, 17, 30, 47). If lysosomes are transported down axons even in small numbers they may fuse with retrogradely transported endosomes with resulting catabolic processing being initiated well before endosomes reach perikarya. Massive involvement of the lysosomal system may deprive axons of this lysosomal input and result in endosomal traffic without lysosomal fusion, i.e. enlarged endosomes. Indeed, the enlarged tubulo- and multi- vesicular profiles seen in spheroids may in fact be enlarged endosomes.

A second possibility is that lysosomal compromise in perikarya might deprive the axon of a key transport molecule such as kinesin or dynein. Indeed, cytoplasmic dynein, the retrograde molecular motor, has been shown to be associated with perikaryal lysosomes and other organelles (37, 44, 49). Finally, another obvious question that deserves consideration: Why are GABAergic neurons particularly vulnerable to axonal spheroid formation? It is widely believed that many types of

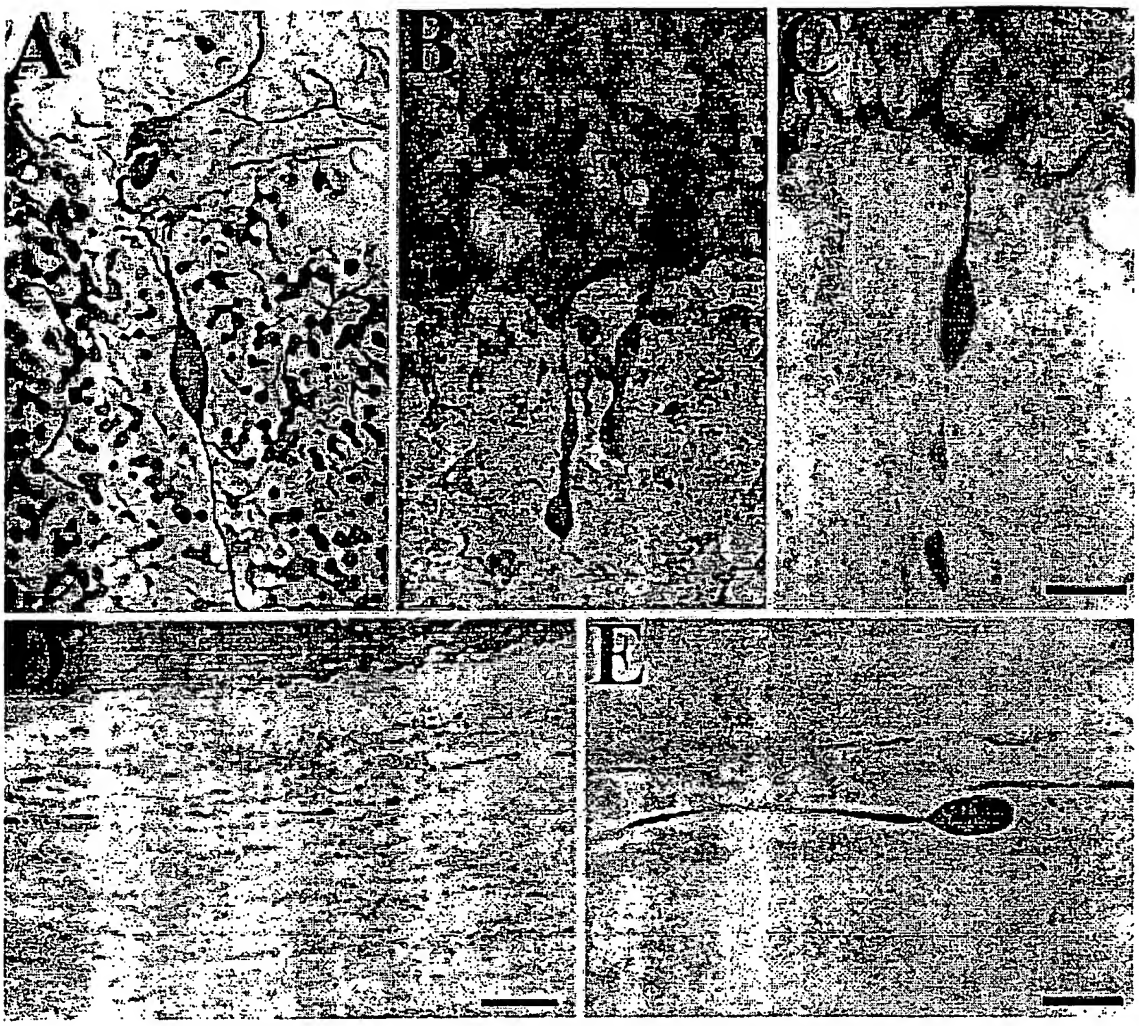


Figure 13. Purkinje cells in storage disease are particularly prone to axonal spheroid formation. As occurs in other types of neurons, spheroids may be seen in proximal (cerebellar cortex, A-C) and distal (cerebellar white matter, D-E) areas of axons. A. Bodian stain; B. GAD immunocytochemistry; C. neurofilament 68 immunocytochemistry; D.-E. GAD immunocytochemistry. Note continuation of distal axon past spheroid in E. Calibration bars equal for C (and A, B) 35μm; D, 110 μm; E, 30 μm.

GABAergic neurons have higher firing rates and metabolic activity than other types of neurons (e.g., see 32). This feature may result in a greater turnover in axonal and synaptosomal components, and thus greater reliance on fully intact anterograde and/or retrograde transport mechanisms.

Spheroids observed in the studies reported above generally have not exceeded 20-30 μm in diameter but even this size likely is capable of causing significant interference with the efficacy or timing of action potential propagation (81). Given recent developments in understanding the crucial role of inhibition in sculpting neuronal activity, such abnormalities would be anticipated to have profound effects on brain function. Indeed, one of the most compelling findings to emerge from these immunocytochemical studies using animal models of GM1 and GM2 gangliosidosis, Niemann-Pick disease, α-mannosidosis, and mucopolysaccharidosis, is the striking correlation between the location and incidence of axonal spheroids and the type and severity of clinical neurological disease (38, 81). Of these diseases only MPS I lacks both significant neuro-

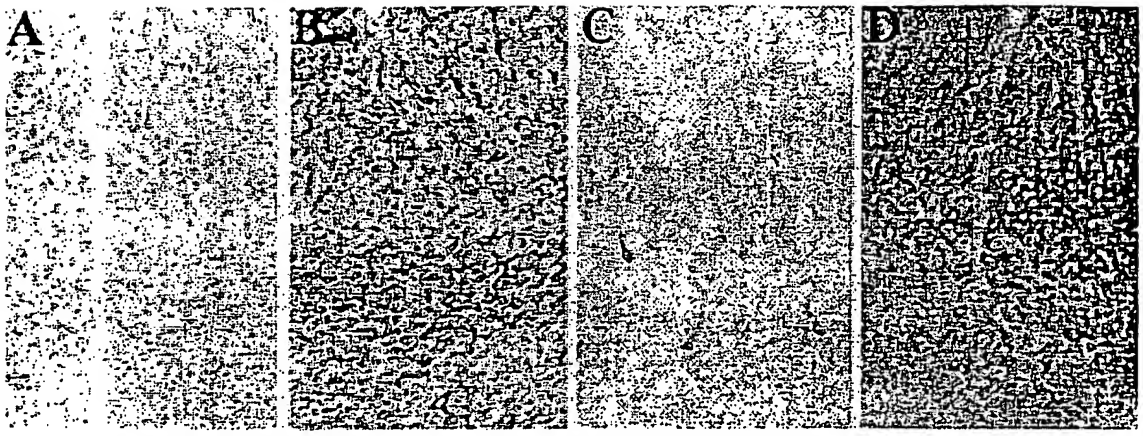


Figure 14. Studies of cerebral cortex in canine NCL show that cortical changes predominate in layer IV. **A.** Nissl stain for overall cellularity; **B.** GFAP stain for astrocytic reactions; **C.** SMI-32 stain for cortical pyramidal neurons; **D.** MAP2 stain for dendrites. Overall there appears to be increased gliosis and neuron loss in the layer IV area. Cortical depths in the 4 sections are matched. All figures shown at 65x magnification.

logical disease and evidence for significant axonal spheroid formation. This is in spite of widespread (near universal) neuronal storage and, indeed, even the presence of spiny meganeurites and ectopic dendritogenesis/synaptogenesis affecting cortical neurons. For the other storage diseases analyzed, the degree of motor system deficits and other neurological findings fully correlate with the incidence of spheroids within the GABAergic cell populations in specific brain regions. It is highly likely, based on these studies, that axonal spheroids account for the generation of more of the clinical neurological disease in these animal models than any other known abnormality, including intraneuronal storage, meganeurite formation and ectopic connectivity and neuron death.

Neuron death. By end stage disease, neuronal cell loss is observed in many types of lysosomal storage disorders. Certain types of neurons, as described for Purkinje cells above, may be vulnerable to cell death earlier in some storage diseases. However, massive death of neurons and accompanying brain atrophy is not a regular feature of the early stages of most types of storage diseases, with one significant exception. This is the disease family known as neuronal ceroid lipofuscinosis or Batten disease. These diseases have been grouped together largely on the basis of similarities in the ultrastructural appearance of the storage material which appears as so-called "curvilinear" or "fingerprint" bodies, or variants thereof. Like other storage disorders, the NCL diseases also occur as infantile, late infantile, juvenile and adult-onset forms. An inability to identify any specific lysosomal hydrolase deficiencies in these diseases, or even to clearly define the storage material (other than as ceroid-lipofuscin), led to these disorders literally being left behind by advances in understanding of most other types of storage disorders in the 1970' and 80's. The discovery that a major storage product in affected cells in the late infantile, juvenile and adult forms of NCL was subunit c of mitochondrial ATP synthase led to their re-classification as lysosomal proteinoses (48). Recent advances defining the gene defects for several of these conditions are also consistent with lysosomal protease deficiencies (27, 65).

The NCL family of diseases is characterized by early death of some types of neurons and accompanying atrophy in specific regions of brain. Most conspicuously affected is the cerebral cortex, with the degree of atrophy rivaling that of Alzheimer's and other neurodegenerative diseases. A conspicuous difference from these adult-onset dementias is, of course, that the atrophy is occurring in childhood for most of the Batten-type disorders.

A major unanswered question is why neurons die in the NCL disorders. Storage is widespread and occurs not only in brain but also in a variety of other tissues. Disease-related cell death, however, predominates only in cerebral cortex, and in some disease forms, in the cerebellum and retina. Other brain regions and tissues show no significant cell loss. Interestingly, the changes in neurons described earlier for other types of storage diseases, ectopic dendritogenesis and axonal spheroid formation, are absent in Batten disease. As shown earlier (Figure 5) some pyramidal neurons do exhibit mega-

neurites but these lack evidence of dendritic features and GM2 ganglioside is not a component of the storage process. Likewise, GABAergic synaptic terminals identified by immunocytochemical techniques may be diminished in number in some brain regions like cerebral cortex or cerebellum, but GAD-positive axonal spheroids are not observed (39, 84).

Selective neuronal vulnerability is a common characteristic of most neurodegenerative diseases and, as described above, the NCL disorders are no exception. One recently hypothesized mechanism to account for this phenomenon in Huntington disease and related disorders is chronic glutamate excitotoxicity (2). According to this view, the neurotransmitter glutamate is deleterious to specific neurons either because it is overabundant or because specific neurons have become vulnerable to its effects due to receptor changes or metabolic defects within the cell (45). This latter view, the so-called "weakened target cell" model, is based on impaired energy production within neurons possessing glutamate receptors (31). Thus, suboptimal mitochondrial function in neurons receiving abundant excitatory glutamatergic input would be anticipated to lead to excessive calcium influx, free radical formation, and cell death. A variety of studies of NCL diseases also suggest mitochondrial abnormalities and free radical damage (54). There is also evidence that subunit c of mitochondrial ATP synthase undergoes abnormal trafficking in NCL cells (11, 12). Animal model studies have supplied evidence for compromise of select inhibitory (GABAergic) circuits in cerebral cortex and cerebellum, and loss of inhibitory inputs likely would exacerbate excitotoxic mechanisms (39, 84).

Evidence supporting the hypothesis of chronic excitotoxicity can be found in the pattern of neuron loss in both the cerebral cortex and cerebellum in canine Batten disease. Changes in specific cerebral cortical layers have been evaluated during disease progression using cytochrome oxidase histochemistry, anti-GFAP and anti-SMI32 antibodies (for astroglial and cortical pyramidal cell labelling, respectively). It has been found that an early and consistent feature of cortical atrophy is reduced cytochrome oxidase activity, a marked astrocytic response, and neuron loss, all of which are centered specifically in midlevel cortex between pyramidal cell layers III and V (Figure 14). The affected layer (IV) in normal brain represents the major receiving zone in cerebral cortex for excitatory thalamocortical inputs. This cortical layer normally demonstrates the highest endogenous cytochrome oxidase staining indicative of a normal, high sustained metabolic activity (94). That cell death and gliosis predominate here in the canine NCL cortex is consistent with intrinsic metabolic demand (and therefore mitochondrial function) being an important player in the process of cell death. Additionally, a subpopulation of scattered GABAergic neurons in NCL cortex exhibit massively enlarge mitochondria, suggestive of a direct compromise in these mitochondria or secondarily-induced physiological reaction unique to this type of neuron (39, 84).

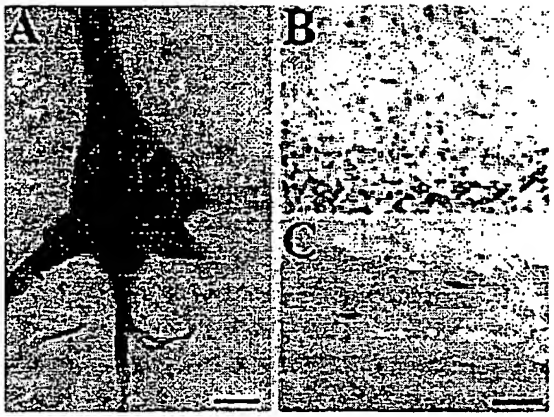


Figure 15. Studies using swainsonine-induced a-mannosidosis reveal that disease-related changes in tissues are not readily reversible post-metabolic correction. **A.** Ectopic dendrites on a Golgi-impregnated cortical pyramdal neuron 2 years after withdrawal of enzyme inhibitor. **B.-C.** Nissl stain of cerebellar cortex revealing loss of Purkinje cells (**B**), and persistence of axonal spheroids (**C**), 4 years post-treatment. Calibration bars equal for A, 10 μm; C (and B) 50 μm.

Similar events also have been reported in the cerebellum in the canine Batten disease model (39). Significant loss of basket cells and basket cell terminals which supply inhibitory inputs to Purkinje cells appears to occur before Purkinje cell loss and cerebellar atrophy. The basket cell population also displays evidence of unique metabolic stress like that of cortical inhibitory neurons: massively enlarged and abnormal-appearing mitochondria. Other neurons exhibit reduced cytochrome oxidase activity.

Reversibility of neuronal changes. An issue of major importance in terms of treating lysosomal storage diseases is that of the potential for reversibility of the cellular pathology characterizing these diseases. In the very early days of the lysosomal disease concept de Duve predicted that correction of these diseases might follow uneventfully if diseased cells had the opportunity and ability to internalize the missing enzyme (10). This is due to the observation that endosomal trafficking

should target the enzyme to the exact organelle, the lysosome, in which it was needed. Numerous studies now demonstrate this capability and a few lysosomal diseases, notably those without CNS involvement, have been effectively treated. Even the neuronal storage disease, α-mannosidosis, has been corrected following bone marrow transplantation (89). In this case it is believed that donor bone marrow-derived microglial cells in brain secrete α-mannosidase which is internalized by neurons with correction of the storage disease.

An issue not addressed in the studies on treatment of genetic studies carried out to date concerns evaluation of the reversibility of cellular changes in brain, most notably ectopic dendritogenesis and axonal spheroid formation. To get at this question an inducible model of neuronal storage has been used, namely that of swainsonine-induced (and reversible) α-mannosidosis (86). Swainsonine is an indolizidine alkaloid derived from locoweed and some other plants. It is a reversible inhibitor of α-mannosidase and if ingested chronically over a 2-4 month period induces a phenotypic replica of genetic α-mannosidosis. Using this model it has been learned that vacuolated lysosomes disappear rapidly (within days) of enzyme replacement (i.e, cessation of administration of the enzyme inhibitor) and neuron swelling and meganeurite formation likewise disappear (92). However, ectopic dendrites and axonal spheroids proved otherwise. Although the incidence of ectopic dendrites was less in animals evaluated months and years after disease correction, some neuritic processes persisted, and indeed appeared to elongate with time in the absence of the disease process (Figure 15). This suggests that once formed, ectopic dendrites may be treated as constituent parts of the neuron as a whole. What makes some ectopic dendrites remain and others disappear is not clear, but stabilization by established synapses is one possibility (78). Likewise, whether such ectopic dendrites and their synaptic input contributes to cortical dysfunction in otherwise metabolically corrected brain remains to be determined.

Axonal spheroids were also found to persist in the swainsonine disease model even years after disease reversal, suggesting that once formed, these structures are not easily eliminated by the neurons even in the face of a normalized perikaryal lysosomal system. Purkinje cell death also appeared to continue after withdrawal of the toxin (Figure 15), possibly due to prolonged deleterious effects of spheroids on these cells, as discussed earlier. In this case the correlation between brain dysfunction and persisting cellular alterations is more apparent. Animals in which storage disease had been induced with swainsonine routinely continued to exhibit cerebellar dysfunction long after complete disappearance of storage from neurons.

Summary

Our knowledge of the genetic and molecular bases of lysosomal storage diseases has expanded dramatically in recent years but a similar indepth understanding of pathogenic mechanisms underlying these diseases has not been achieved. The pathogenic cascade of storage diseases can be exceedingly complex and involve not only secondary lysosomal storage but also a host of nonlysosomal events that may impact the cell in diverse ways. As a consequence, storage diseases are far more complex than their simple origins as single enzyme deficiencies might suggest. As recently argued by Kunihiko Suzuki, one of the pioneers in the study of biochemical and molecular bases of lysosomal diseases (68), the future of studies on storage diseases, to a very large degree, lies in the understanding of their biology. The rewards of these studies will be not only a better grasp on the full range of treatment strategies for these disorders, but too, greater insight into the workings of normal neurons and other cells through study of their kind in the absence of a single enzyme. Garrod's reflection (14) on William Harvey's original view of the study of "rarer forms of disease" is as apt today as three centuries ago. That is, by understanding the details of the pathogenic mechanisms of these diseases, we will learn much about the working of normal cells.

Acknowledgements

I thank M. Zervas and Drs. D. Siegel and K. Dobrenis for numerous helpful discussions and for their collaborative efforts. This work is supported by grants from the NIH and the Ara Parseghian Medical Research Foundation.

References

1. Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson J (1994) The Molecular Biology of the Cell. 3rd Edition. Garland: New York
2. Beal MF (1992) Does impairment of energy metabolism result in excitotoxic neuronal death in neurodegenerative disease? *Ann Neurol* 31: 119-130
3. Bevan AP, Drake PG, Bergeron JJM, Posner BI (1996) Intracellular signal transduction: The role of endosomes. *TEM* 7: 13-21
4. Bielschowsky M (1932) Histopathology of nerve cells. In: Cytology and Cellular Pathology of the Nervous System, Penfield W (ed.), Paul B. Hoeber: New York

5. Bremer E (1994) Glycosphingolipids as effectors of growth and differentiation. *Curr Top Membranes: Cell Lipids* 40: 387-411
6. Broadwell RD, Oliver C, Brightman MW (1980) Neuronal transport of acid hydrolases and peroxidase within the lysosomal system of organelles: Involvement of agranular reticulum-like cisterns. *J Comp Neurol* 190: 519-532
7. Brown D, Thrall MA, Walkley SU, Wurzelmann S, Wenger DA, Allison RW, Just CA (1996) Metabolic abnormalities in feline Niemann-Pick disease type C heterozygotes. *J Inher Metab Dis* 19: 319-330
8. Chakraborty M, Anderson GM, Chakraborty A, Chatterjee D (1993) Accumulation of high level of pp60 c-srcN is an early event during GM3 -antibody mediated differentiation of neuro-2a neuroblastoma cells. *Brain Res* 625: 197-202
9. Conzelmann E, Sandhoff K (1983/84) Partial enzyme deficiencies: residual activities and development of neurological disorders. *Dev Neurosci* 6: 58-71
10. de Duve C (1964) From cytases to lysosomes. *Fed Proc* 23: 1045-1049
11. Ezaki J, Wolfe LS, Ishido K, Kominami E (1995) Abnormal degradative pathway of mitochondrial ATP synthase subunit c in late infantile neuronal ceroid lipofuscinosis (Batten disease). *Am J Med Genet* 57: 254-259
12. Ezaki JR, Wolfe LS, Kominami E (1996) Specific delay in the degradation of mitochondrial ATP synthase subunit c in late infantile neuronal ceroid-lipofuscinosis is derived from cellular proteolytic dysfunction rather than structural alteration of subunit c. *J Neurochem* 67: 1677-1687
13. Ferrari G, Anderson BL, Stephens RM, Kaplan DR, Greene LA (1995) Prevention of apoptotic neuronal death by GM1 ganglioside. *J Biol Chem* 270: 3074-3080
14. Garrod A (1928) The lessons of rare maladies. *Lancet* 1: 1055-1059
15. Garrod AE (1909) Inborn Errors of Metabolism. Oxford University Press: Oxford
16. Gatzinsky KP, Berthold C-H (1990) Lysosomal activity at nodes of Ranvier during retrograde axonal transport of horseradish peroxidase in alpha-motor neurons of the cat. *J Neurocytol* 20: 989-1002
17. Gatzinsky KP, Berthold C-H, Corneliuson O (1988) Acid phosphatase activity at nodes of Ranvier in alpha-motor and dorsal root ganglion neurons of the cat. *J Neurocytol* 17: 531-544
18. Goodman LA, Livingston PO, Walkley SU (1991) Ectopic dendrites occur only on cortical pyramidal cells containing elevated GM2 ganglioside in α-mannosidosis. *Proc Natl Acad Sci USA* 88: 11330-11334
19. Goodman LA, Walkley SU (1996) GM2 ganglioside is associated with cortical neurons undergoing active dendritogenesis during normal brain development. *Dev Brain Res* 93: 162-171
20. Gravel RA, Clarke JTR, Kaback MM, Mahuran D, Sandhoff K and Suzuki K (1995) The GM2 gangliosides. In: The Metabolic and Molecular Bases of Inherited Disease, Scriver CR, Beaudet AL, Sly WS, Valle D (eds.), Chapter 92, pp. 2839-2879, McGraw-Hill: New York
21. Griffiths G, Gruenberg J (1991) The arguments for preexisting early and late endosomes. *Trends Cell Biol* 1: 5-9
22. Hakomori S (1995) Role of gangliosides in transmembrane signaling and cell recognition. In: Biology of the Sialic Acids, Rosenberg A (ed.), pp. 243-259, Plenum Press Publishers: New York
23. Hers HG (1965) Progress in gastroenterology: Inborn lysosomal diseases. *Gastroenterology* 48: 625-633
24. Hers HG and Van Hoof F (1973) Lysosomes and Storage Diseases. Academic Press: New York
25. Hirschberg, K Zisling R, van Echten-Deckert, G, Futerman AH (1996) Ganglioside synthesis during the development of neuronal polarity. *J Biol Chem* 271 :14876-14882
26. Hoekstra D, Kok JW (1992) Trafficking of glycosphingolipids in eukaryotic cells; sorting and recycling of lipids. *Biochim Biophys Acta* 1113: 277-294
27. Hofmann SL, Lee LA, Lu J-Y, Verkruyse LA (1997) Palmitoyl-protein thioesterase and the molecular pathogenesis of infantile neuronal ceroid lipofuscinosis. *Neuropediatrics* 28: 27-30
28. Hollenbeck PJ (1993) Products of endocytosis and autophagy are retrieved from axons by regulated retrograde organelle transport. *J Cell Biol* 121: 305-315
29. Holtzman E. (1989) Lysosomes. Plenum Press: New York
30. Holtzman E, Novikoff AB (1965) Lysosomes in the rat sciatic nerve following crush. *J Cell Biol* 27: 651-668
31. Horowski R, Wachtel H, Turski L, Löschmann P-A (1994) Glutamate excitotoxicity as a possible pathogenetic mechanism in chronic neurodegeneration. In: Neurodegenerative Diseases, Calne DB (ed.), Chapter 11, pp. 163-175, WB Saunders: Philadelphia
32. Houser CR, Vaughn JE, Hendry SHC, Jones EG, Peters A (1984) GABA neurons in the cerebral cortex. In: Cerebral Cortex: Functional Properties of Cortical Cells, Jones EG, Peter A (eds), Volume 2, pp. 63-89, Plenum Press: New York
33. Karabelas AB , Walkley SU (1985) Altered patterns of evoked synaptic activity in cortical pyramidal neurons in feline ganglioside storage disease. *Brain Res* 339: 329-336
34. Kornfeld S, Mellman I (1989) The biogenesis of lysosomes. *Annu Rev Cell Biol* 5: 483-526
35. Lake B (1997) Lysosomal and peroxisomal disorders. Greenfield's Neuropathology, Graham DI, Lantos PL (eds.) Volume 1, Chapter 11, pp. 657-753, Arnold: London
36. Ledeen RW (1985) Biology of gangliosides: Neuritogenic and neuronotrophic properties. In: Neurobiology of Gangliosides,Gorio A, Haber B (eds.), pp. 147-159, Alan R. Liss, Inc. Publisher: New York
37. Lin SXH, Collins CA (1992) Immunolocalization of cytoplasmic dynein to lysosomes in cultured cells. *J Cell Sci* 101: 125-137
38. March PA, Thrall MA, Wurzelmann S, Brown D, Walkley SU (1997) Dendritic and axonal abnormalities in feline Niemann-Pick disease type C. *Acta Neuropathol (Berl)* 94: 164-172

39. March PA, Walkley SU, Wurzelmann S (1995) Morphological alterations in neocortical and cerebellar GABAergic neurons in canine Batten's disease. *Am J Med Genet* 57: 204-212

40. Murphy RF (1991) Maturation models for endosome and lysosome biogenesis. *Trends Cell Biol* 1: 77-82

41. Mutoh T, Tokuda A, Miyadai T, Hamaguchi M, Fujiki N (1995) Ganglioside GM1 binds to the TRK protein and regulates receptor function. *Proc Natl Acad Sci USA* 92: 5087-5091

42. Neufeld E (1991) Lysosomal storage diseases. *Annu Rev Biochem* 60: 257-280

43. Nixon RA, Cataldo AM (1995) The endosomal-lysosomal system of neurons: new roles. *Trends Neurosci* 18: 489-496

44. Noda Y, Nakata T, Hirokawa N (1993) Localization of dynamin: Widespread distribution in mature neurons and association with membranous organelles. *Neuroscience* 55: 113-127

45. Novelli A, Reilly JA, Lysko PG, Henneberry, RC (1988) Glutamate becomes neurotoxic via the N-methyl-D-aspartate receptor when intracellular energy levels are reduced. *Brain Res* 451: 205-212

46. Novikoff AB (1973) Lysosomes : A personal account. In: Lysosomes and Storage Diseases, Hers HG, van Hoof F (eds.), Chapter 1, pp. 2-41, Academic Press: New York

47. Overly CC, Lee K, Berthiaume E, Hollenbeck PJ (1995) Quantitative measurement of intraorganelle pH in the endosomal-lysosomal pathway in neurons by using ratiometric imaging with pyranine. *Proc Natl Acad Sci USA* 92: 3156-3160

48. Palmer DN, Fearnley IM, Walker JE, Hall NA, Lake BD, Wolfe LS, Haltia M, Martinus RD, Jolly RD (1992) Mitochondrial ATP synthase subunit c storage in the ceroid lipofuscinoses (Batten disease). *Am J Med Genet* 42: 561-567

49. Parton RG, Dotti CG (1993) Cell biology of neuronal endocytosis. *J Neurosci Res* 36: 1-9

50. Parton RG, Simons K, Dotti CG (1992) Axonal and dendritic endocytic pathways in cultured neurons. *J Cell Biol* 119: 123-137

51. Pentchev PG, Comly ME, Kruth HS, Vanier MT, Wenger DA, Patel S, Brady RO (1985) A defect in cholesterol esterification in Niemann-Pick disease (type C) patients. *Proc Natl Acad Sci USA* 82: 8247-8251

52. Purpura DP, Suzuki K (1976) Distortion of neuronal geometry and formation of aberrant synapses in neuronal storage disease. *Brain Res* 116: 1-21

53. Rabin SJ, Mocchetti I (1995) GM1 ganglioside activates the high-affinity nerve growth factor receptor TRKA. *J Neurochem* 65: 347-354

54. Rider JA, Dawson G, Siakotos AN (1992) Perspective of biochemical research in the neuronal ceroid-lipofuscinosis. *Am J Med Genet* 42: 519-524

55. Sachs B (1887) On arrested cerebral development, with special reference to its cortical pathology. *J Nervous Ment Dis* 14: 541-553

56. Sachs B (1903) On amaurotic family idiocy. A disease chiefly of the gray matter of the central nervous system. *J Nervous Ment Dis* 30: 1-13

57. Sachs B, Strauss I (1910) The cell changes in amaurotic family idiocy. *J Exp Med* 12: 685-695

58. Sandhoff K, van Echten G (1994) Ganglioside metabolism: Enzymology, topology, and regulation. *Prog Brain Res* 101: 17-30

59. Sandhoff K, Klein A (1994) Intracellular trafficking of glycosphingolipids: Role of sphingolipid activator proteins in the topology of endocytosis and lysosomal digestion. *FEBS Lett* 346: 103-107

60. Sandhoff K, Kolter T (1996) Topology of glycosphingolipid degradation. *Trends Cell Biol* 6: 98-103

61. Sandhoff K, Schwarzmann G (1989) Dynamics of gangliosides in neuronal membranes. In: Progress in Zoology, Voume 37: Fundamentals of Memory Formation; Neuronal Plasticity and Brain Function., Rahmann H (ed.), pp. 229-239, VCH Publishers: New York

62. Schwarzmann G, Sandhoff K (1990) Metabolism and intracellular transport of glycosphingolipids. *Biochemistry* 29: 10865-10870

63. Scriver CR, Beaudet AL, Sly WS and Valle D (eds.) (1995) Lysosomal enzymes. The Metabolic and Molecular Bases of Inherited Disease, Chapters 76-92, pp. 2427-2879, McGraw-Hill: New York

64. Siegel DA, Walkley SU (1994) Growth of ectopic dendrites on cortical pyramidal neurons in neuronal storage diseases correlates with abnormal accumulation of GM2 ganglioside. *J Neurochem* 62: 1852-1862

65. Sleat DE, Donnelly, RJ, Lackland H, Liu C, Sohar I, Pullarkat RK, Lobel P (1997) Association of mutations in a lysosomal protein with classical late-infantile neuronal ceroid -lipofuscinosis. *Science* 277: 1802-1805

66. Smith RS (1980) The short term accumulation of axonally transported organelles in the region of localized lesions of single myelinated axons. *J Neurocytol* 9: 39-65

67. Suzuki K (1976) Neuronal storage diseases: A review. In: Progress in Neuropathology, Zimmerman HM (ed.), Volume 3, pp. 173-202, Grune & Straton: New York

68. Suzuki K (1994) Molecular genetics of Tay-Sachs and related disorders: a personal account. *J Neuropathol Exp Neurol* 53: 344-350

69. Swanson JA, Watts C (1995) Macropinocytosis. *Trends Cell Biol* 5: 424-428

70. Terry R, Weiss M (1963) Studies in Tay-Sachs disease II. Ultrastructure of the cerebrum. *J Neuropathol Exp Neurol* 22: 18-55

71. Tsukita S, Ishikawa H (1980) The movement of membranous organelles in axons: electron microscopic identification of anterogradely and retrogradely transported organelles. *J Cell Biol* 84: 513-530

72. van Echten G, Sandhoff K (1993) Ganglioside metabolism - enzymology, topology and regulation. *J Biol Chem* 268: 5341-5344

73. van Meer G, Burger KNJ (1992) Sphingolipid trafficking - sorted out? *Trends Cell Biol* 2: 332-337

74. Wada I, Lai WH, Posner BI, Bergeron JJM (1992) Association of the tyrosine phosphorylated epidermal growth factor receptor with a 55-kD tyrosine phosphorylated protein at the cell surface and in endosomes. *J Cell Biol* 16: 321-330

75. Walkley SU (1987) Further studies on ectopic dendrite growth and other geometrical distortions of neurons in feline GM1 gangliosidosis. *Neuroscience* 21: 313-331

76. Walkley SU (1988) Pathobiology of neuronal storage disease. *Int Rev Neurobiol* 29: 191-244

77. Walkley SU (1995) Pyramidal neurons with ectopic dendrites in storage diseases contain elevated levels of GM2 ganglioside. *Neuroscience* 68: 1027-1035

78. Walkley SU, Wurzelmann, S (1995) Alterations in synaptic connectivity in cerebral cortex in neuronal storage diseases. *Ment Retard Dev Disabilities Rev* 1: 183-192

79. Walkley SU, Baker HJ (1984) Sphingomyelin lipidosis in a cat. II. Golgi studies. *Acta Neuropathol (Berl)* 65: 138-144

80. Walkley SU, Baker HJ, Rattazzi MC (1990) Initiation and growth of ectopic neurites and meganeurites during postnatal brain development in ganglioside storage disease. *Dev Brain Res* 51: 167-178

81. Walkley SU, Baker HJ, Rattazzi MC, Haskins ME, Wu J-Y (1991) Neuroaxonal dystrophy in neuronal storage disorders: Evidence for major GABAergic neuron involvement. *J Neurol Sci* 104: 1-8

82. Walkley SU, Blakemore WF, Purpura DP (1982) Alterations in neuron morphology in feline mannosidosis: A Golgi study. *Acta Neuropathol (Berl)* 53: 75-79

83. Walkley SU, Haskins ME, Shull RM (1988) Alterations in neuron morphology in mucopolysaccharide storage disorders. *Acta Neuropathol (Berl)* 75: 611-620

84. Walkley SU, March PA, Schroeder CE, Wurzelmann S, Jolly RD (1995) Pathogenesis of brain dysfunction in Batten's disease. *Am J Med Genet* 57: 196-203

85. Walkley SU, Pierok AL (1986) Ferric ion-ferrocyanide staining in ganglioside storage disease establishes that meganeurites are of axon hillock origin and distinct from axonal spheroids. *Brain Res* 382: 379-386

86. Walkley SU, Siegel DA (1989) Comparative studies of the CNS in swainsonine-induced and inherited feline α-mannosidosis. In: Swainsonine and Related Glycosidase Inhibitors, James LF, Elbein AD, Molyneux RJ, Warren CD (eds.), pp. 57-75, Iowa State University Press: Ames

87. Walkley SU, Siegel DA, Dobrenis, K (1995) GM2 ganglioside and pyramidal neuron dendritogenesis. *Neurochem Res* 20: 1287-1299

88. Walkley SU, Siegel DA, Wurzelmann S (1988) Ectopic dendritogenesis and associated synapse formation in swainsonine-induced neuronal storage disease. *J Neurosci* 8: 445-457

89. Walkley SU, Thrall MA, Dobrenis K, Huang M, March P, Siegel D, Wurzelmann S (1994) Bone marrow transplantation corrects the enzyme defect of the central nervous system in a lysosomal storage disease. *Proc Natl Acad Sci USA* 91: 2970-2974

90. Walkley SU, Wurzelmann S, Purpura DP (1981) Ultrastructure of neurites and meganeurites of cortical pyramidal neurons in feline gangliosidosis as revealed by the combined Golgi-EM technique. *Brain Res* 211: 393-398

91. Walkley SU, Wurzelmann S, Rattazzi MC, Baker, HJ (1990) Distribution of ectopic neurite growth and other geometrical distortions of neurons in feline GM2 gangliosidosis. *Brain Res* 510: 63-73

92. Walkley SU, Wurzelmann S, Siegel DA (1987) Ectopic axon hillock-associated neurite growth is maintained in metabolically reversed swainsonine-induced neuronal storage disease. *Brain Res* 410: 89-96

93. Williams RS, Lott, IT, Ferrante RJ, Caviness VS (1977) The cellular pathology of neuronal ceroid-lipofuscinosis. *Arch Neurol* 34: 298-305

94. Wong-Riley MTT (1989) Cytochrome oxidase: an endogenous metabolic marker for neuronal activity. *Trends Neurosci* 12: 94-101

95. Yates AJ (1986) Gangliosides in the nervous system during development and regeneration. *Neurochem Pathol* 5: 309-329

96. Yu RK (1994) Developmental regulation of ganglioside metabolism. *Prog Brain Res* 101: 31-44

97. Zeller CB, Marchase RB (1992) Gangliosides as modulators of cell function. *Am J Physiol* C1341-C1355

98. Zeman W, Donahue S (1963) Fine structure of the lipid bodies in juvenile amaurotic idiocy. *Acta Neuropath (Berl)* 3: 144-149

Field: **Title,** Limits: **Publication Date to 2001/10/29**

Display Abstract Show: 50 Sort Send to Text

Items 1-48 of 48

One page.

1: J Inherit Metab Dis. 2001;24 Suppl 2:47-51; discussion 45-6. Related Articles, Links

kluweronline
Full text article

Variable clinical presentation in lysosomal storage disorders.

Beck M.

Children's Hospital, University of Mainz, Germany. Beck@kinder.klinik.uni-mainz.de

Extensive clinical heterogeneity is seen in lysosomal storage disorders, regarding the age of onset and severity of symptoms, the organs involved, and effects on the central nervous system. A broad phenotypic spectrum is seen, for example, in mucopolysaccharidosis type I (Hurler/Scheie disease), Gaucher disease, the several forms of GM2-gangliosidosis and the different manifestations of beta-galactosidase deficiency (GM1-gangliosidosis and Morquio disease type B). Variable clinical expression of the same enzyme defect is not well understood. The presence of different mutations is only part of the explanation, as intrafamilial variability is observed in many cases. Other mechanisms, for example the effect of specific activators, may also have an influence on phenotype.

Publication Types:
- Review
- Review, Tutorial

PMID: 11758678 [PubMed - indexed for MEDLINE]

2: Chem Rev. 2000 Dec 13;100(12):4683-96. Related Articles, Links

ACS PUBLICATIONS

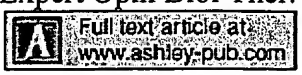
Inhibition of glycosphingolipid biosynthesis: application to lysosomal storage disorders.

Butters TD, Dwek RA, Platt FM.

Oxford Glycobiology Institute, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, U.K.

PMID: 11749362 [PubMed - as supplied by publisher]

3: Expert Opin Biol Ther. 2001 Sep;1(5):857-67. Related Articles, Links



Gene therapy for lysosomal storage disorders.

Barranger JM, Novelli EA.

University of Pittsburgh, Department of Human Genetics, PA, USA.
jbarrang@helix.hgen.pitt.edu

The lysosomal storage disorders (LSD) are monogenic inborn errors of metabolism with heterogeneous pathophysiology and clinical manifestations. In the last decades, these disorders have been models for the development of molecular and cellular therapies for inherited metabolic diseases. Studies in preclinical in vitro systems and animal models have allowed the successful development of bone marrow transplantation (BMT) and enzyme replacement therapy (ERT) as therapeutic options for several LSDs. However, BMT is limited by poor donor availability and high morbidity and mortality, and ERT is not a life-long cure. Moreover, the neuropathology present in many LSDs responded poorly, if at all, to these treatments. Therefore, gene therapy is an attractive therapeutic alternative. Gene therapy strategies for LSDs have employed ex vivo gene transduction of cellular targets with subsequent transplantation of the enzymatically corrected cells, or direct in vivo delivery of the viral vectors. Oncoretroviral vectors and more recently adeno associated vectors (AAV) and lentiviral vectors have been extensively tested, with some success. This review summarises the main gene therapy strategies which have been employed or are under development for both non-neurological and neuronopathic LSDs. Some of the in vitro and in vivo preclinical studies presented herein have provided the rationale for a gene therapy clinical trial for Gaucher disease Type I.

Publication Types:
- Review
- Review, Tutorial

PMID: 11728220 [PubMed - indexed for MEDLINE]

4: Eur J Paediatr Neurol. 2001;5 Suppl A:73-9. Related Articles, Links

Tripeptidyl-peptidase I in neuronal ceroid lipofuscinoses and other lysosomal storage disorders.

Wisniewski KE, Kida E, Walus M, Wujek P, Kaczmarski W, Golabek AA.

Department of Pathological Neurobiology, New York State Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Road, Staten Island, NY 10314, USA.

The classic late infantile form of neuronal ceroid lipofuscinosis (CLN2, cLINCL) is associated with mutations in the gene encoding tripeptidyl-peptidase I (TPP-I), a lysosomal aminopeptidase that cleaves off tripeptides from the free N-termini of oligopeptides. To date over 30 different mutations and 14 polymorphisms associated with CLN2 disease process have been identified. In the present study, we analysed the molecular basis of 15

different mutations of TPP-I by using immunocytochemistry, immunofluorescence, Western blotting, enzymatic assay and subcellular fractionation. In addition, we studied the expression of TPP-I in other lysosomal storage disorders such as CLN1, CLN3, muccopolysaccharidoses and GM1 and GM2 gangliosidoses. Our study shows that TPP-I is absent or appears in very small amounts not only in cLINCL subjects with mutations producing severely truncated protein, but also in individuals with missense point mutations, which correlates with loss of TPP-I activity. Of interest, small amounts of TPP-I were detected in lysosomal fraction from fibroblasts from cLINCL subject with protracted form. This observation suggests that the presence of small amounts of TPP-I in lysosomes is able to delay significantly CLN2 disease process. We also show that TPP-I immunoreactivity is increased in the brain tissue of CLN1 and CLN3 subjects, stronger in glial cells and macrophages than neurons. Less prominent increase of TPP-I staining was found in muccopolysaccharidoses and GM1 and GM2 gangliosidoses. These data suggest that TPP-I participates in lysosomal turnover of proteins in pathological conditions associated with cell/tissue injury.

PMID: 11589013 [PubMed - indexed for MEDLINE]

5: Ment Retard Dev Disabil Res Rev. 2001;7(3):190-9. Related Articles, Links

Systematic approach to the diagnosis of lysosomal storage disorders.

Weibel TD, Brady RO.

Developmental and Metabolic Neurology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20892-1260, USA.

Disorders that arise as a result of lysosomal dysfunction represent some of the most challenging diagnostic problems in medicine. Not only are these disorders infrequently seen, but they may also present with signs and symptoms that mimic perinatal injury, food intolerance, or the sequellae of neonatal infection. Misidentification can lead to significant delay in diagnosis. Ironically, as the prevailing economic climate places increasing time constraints on practicing physicians, medical research is providing treatment strategies and management techniques that are most effective if applied early in the course of the disease. Most lysosomal storage disorders can now be definitively diagnosed once the signs are recognized. In many cases the benefits of early diagnosis, enlightened management, and appropriate referral are considerable. The aim of this paper is to demystify this elusive class of diseases, to promote clinical vigilance in their detection, and to provide a systematic approach to diagnosis when clinical suspicion is aroused. Copyright 2001 Wiley-Liss, Inc.

Publication Types:
- Review
- Review, Tutorial

PMID: 11553935 [PubMed - indexed for MEDLINE]

6: Curr Opin Mol Ther. 2001 Aug;3(4):399-406. Related Articles, Links

Gene therapy for lysosomal storage disorders.

Yew NS, Cheng SH.

Genzyme Corporation, Framingham, MA 01701-9322, USA. nelson.yew@genzyme.com

Deficiencies in one or several of the numerous degradative enzymes that reside in the lysosome often result in one of many clinically severe diseases, almost all of which have no currently available therapy. Although bone marrow transplantation, enzyme replacement and substrate inhibition therapies are being considered, gene therapy represents an increasingly attractive approach, particularly for those lysosomal storage diseases with neurological manifestations. This review summarizes the most recent advances in developing gene therapies for this large and heterogeneous group of disorders.

Publication Types:
- Review
- Review, Tutorial

PMID: 11525564 [PubMed - indexed for MEDLINE]

☐ 7: Southeast Asian J Trop Med Public Health. 1999;30 Suppl 2:111-3. Related Articles, Links

Pilot neonatal screening program for lysosomal storage disorders, using lamp-1.

Ranierri E, Gerace RL, Ravenscroft EM, Hopwood JJ, Meikle PJ.

Department of Chemical Pathology, Women's and Children's Hospital, North Adelaide, Australia.

We have demonstrated that the lysosome associated membrane protein (LAMP-1) is elevated in plasma from approximately 70% of lysosomal storage disorder patients. As part of the development of a newborn screening program for lysosomal storage disorders we have developed a first tier screening assay based upon the level of LAMP-I in blood spots taken from newborn Guthrie cards. To determine the effectiveness of the first-tier marker a prospective pilot Guthrie neonatal screening program for the identification of LSD was commenced in April 1998. Prior to commencement of the pilot program ethical approval was obtained and information leaflets regarding the neonatal screening of LSD were distributed to parents at the time of their infant's Guthrie collection. The LAMP-1 assay utilizes a chicken polyclonal and a mouse monoclonal in a sandwich time resolved fluorescent immunoassay. LAMP-1 blood-spot calibrators and quality control specimens were developed and shown to be stable and reproducible. To date 11,183 infants have been screened using LAMP-1. The population distribution is described with a median and 98th percentile of 220pg/l whole blood and 483microg/l whole blood respectively. Acceptable CV% for intra and inter assay of 8.9% and 10% respectively were obtained.

PMID: 11400745 [PubMed - indexed for MEDLINE]

8: Southeast Asian J Trop Med Public Health. 1999;30 Suppl 2:104-10. Related Articles, Links

Newborn screening for lysosomal storage disorders.

Meikle PJ, Ranieri E, Ravenscroft EM, Hua CT, Brooks DA, Hopwood JJ.

Department of Chemical Pathology, Women's and Children's Hospital, North Adelaide, Australia. pmeikle@medicine.adelaide.edu.au

Lysosomal storage disorders (LSD) represent a group of over 40 distinct genetic diseases with a total incidence of approximately 1:7,000 births. Bone marrow transplantation and enzyme replacement therapy are currently in use for the treatment of some disorders and new forms of enzyme and gene replacement therapy are actively being researched. The effectiveness of these therapies, particularly for the LSD involving the central nervous system and bone pathology, will rely heavily upon the early diagnosis and treatment of the disorder, before the onset of irreversible pathology. In the absence of a family history the only practical way to detect these disorders will be by a newborn screening program. One common feature of these disorders is an increase in the number and size of lysosomes within the cell from approximately 1% to as much as 50% of total cellular volume. Associated with this, is a corresponding increase in some lysosomal proteins. We propose that the measurement of one or more of these proteins in blood spots taken from Guthrie cards, will form the basis of a newborn screening program, for the detection of all LSD. We have identified a number of lysosomal proteins as potential markers for LSD. The level of these proteins has been determined in blood spots taken from Guthrie cards and in plasma samples from over 300 LSD affected individuals representing 25 disorders. Based on these results we have proposed a strategy for a newborn screening program involving a two tier system, utilizing time resolved fluorescence immunoquantification of the protein markers in the first tier, followed by tandem mass spectrometry for the determination of stored substrates in the second tier assays.

PMID: 11400743 [PubMed - indexed for MEDLINE]

9: Clin Chem. 2000 Sep;46(9):1318-25. Related Articles, Links

FREE full text article at www.clinchem.org

Determination of acid alpha-glucosidase protein: evaluation as a screening marker for Pompe disease and other lysosomal storage disorders.

Umapathysivam K, Whittle AM, Ranieri E, Bindloss C, Ravenscroft EM, van Diggelen OP, Hopwood JJ, Meikle PJ.

Lysosomal Diseases Research Unit and State Screening Services, Department of Chemical Pathology, Women's and Children's Hospital, 72 King William Rd., North Adelaide, South Australia 5006, Australia.

BACKGROUND: In recent years, there have been significant advances in the development of enzyme replacement and other therapies for lysosomal storage disorders (LSDs). Early diagnosis, before the onset of irreversible pathology, has been demonstrated to be critical for maximum efficacy of current and proposed therapies. In the absence of a family history, the presymptomatic detection of these disorders ideally can be achieved through a newborn screening program. One approach to the development of such a program is the identification

of suitable screening markers. In this study, the acid alpha-glucosidase protein was evaluated as a marker protein for Pompe disease and potentially for other LSDs. METHODS: Two sensitive immunoquantification assays for the measurement of total (precursor and mature) and mature forms of acid alpha-glucosidase protein were used to determine the concentrations in plasma and dried blood spots from control and LSD-affected individuals. RESULTS: In the majority of LSDs, no significant increases above control values were observed. However, individuals with Pompe disease showed a marked decrease in acid alpha-glucosidase protein in both plasma and whole blood compared with unaffected controls. For plasma samples, this assay gave a sensitivity of 95% with a specificity of 100%. For blood spot samples, the sensitivity was 82% with a specificity of 100%. CONCLUSIONS: This study demonstrates that it is possible to screen for Pompe disease by screening the concentration of total acid alpha-glucosidase in plasma or dried blood spots.

PMID: 10973860 [PubMed - indexed for MEDLINE]

10: J Am Soc Nephrol. 2000 Aug;11(8):1542-7. Related Articles, Links

Full text article at www.jasn.org

Gene therapy for lysosomal storage disorders with neuropathology.

Ioannou YA.

Department of Human Genetics, Gene Therapy and Molecular Medicine, The Mount Sinai School of Medicine, New York, NY 10029-6574, USA. ioanny01@doc.mssm.edu

Publication Types:
- Review
- Review, Tutorial

PMID: 10906169 [PubMed - indexed for MEDLINE]

11: Clin Chem. 2000 Feb;46(2):167-74. Related Articles, Links

FREE full text article at www.clinchem.org

Saposins A, B, C, and D in plasma of patients with lysosomal storage disorders.

Chang MH, Bindloss CA, Grabowski GA, Qi X, Winchester B, Hopwood JJ, Meikle PJ.

Lysosomal Diseases Research Unit, Department of Chemical Pathology, Women's and Children's Hospital, 72 King William Road, North Adelaide, South Australia 5006, Australia.

BACKGROUND: Early diagnosis of lysosomal storage disorders (LSDs), before the onset of irreversible pathology, will be critical for maximum efficacy of many current and proposed therapies. To search for potential markers of LSDs, we measured saposins A, B, C, and D in patients with these disorders. METHODS: Four time-delayed fluorescence

immunoquantification assays were used to measure each of the saposins in plasma from 111 unaffected individuals and 334 LSD-affected individuals, representing 28 different disorders. RESULTS: Saposin A was increased above the 95th centile of the control population in 59% of LSD patients; saposins B, C, and D were increased in 25%, 61%, and 57%, respectively. Saposins were increased in patients from several LSD groups that in previous studies did not show an increase of lysosome-associated membrane protein-1 (LAMP-1). CONCLUSION: Saposins may be useful markers for LSDs when used in conjunction with LAMP-1.

PMID: 10657372 [PubMed - indexed for MEDLINE]

☐ **12:** Adv Pediatr. 1999;46:409-40. Related Articles, Links

Hydrops fetalis: lysosomal storage disorders in extremis.

Stone DL, Sidransky E.

Clinical Neuroscience Branch, National Institute of Mental Health, Bethesda, Maryland, USA.

In recent years there has been an increased recognition that hydrops fetalis may be an extreme presentation of many of the lysosomal storage disorders. Hydrops fetalis, the excessive accumulation of serous fluid in the subcutaneous tissues and serous cavities of the fetus, has many possible etiologies, providing a diagnostic challenge for the physician. Ten different lysosomal storage disorders have now been diagnosed in infants with hydrops fetalis, including mucopolysaccharidosis (MPS) VII and IVA, type 2 Gaucher disease, sialidosis, GMI gangliosidosis, galactosialidosis, Niemann-Pick disease type C, disseminated lipogranulomatosis (Farber disease), infantile free sialic acid storage disease (ISSD), and mucolipidosis II (I-cell disease). Frequently, these inborn errors of metabolism are recognized only after the unfortunate recurrence of hydrops fetalis in several pregnancies of a family. Making the diagnosis relies on the physician having a high index of suspicion and ordering appropriate testing, which can often be performed prenatally. In several of these disorders, including MPS VII, infantile galactosialidosis, type 2 Gaucher disease, and ISSD, hydrops fetalis is a relatively common presentation. A greater physician awareness of hydrops fetalis as a presentation of lysosomal disease will facilitate establishing a diagnosis in cases that would have previously been considered idiopathic and will enable a better estimation of the incidence of this association. Lysosomal disorders are among the few causes of nonimmune hydrops fetalis in which an accurate recurrence risk can be ascertained. With an early and accurate diagnosis, genetic counseling and family planning can be offered in these difficult cases.

Publication Types:
- Review
- Review, Tutorial

PMID: 10645471 [PubMed - indexed for MEDLINE]

☐ **13:** Pediatr Res. 1999 Nov;46(5):501-9. Related Articles, Links

Alpha-mannosidosis in the guinea pig: a new animal model for lysosomal storage disorders.

Crawley AC, Jones MZ, Bonning LE, Finnie JW, Hopwood JJ.

Department of Chemical Pathology, Women's and Children's Hospital, North Adelaide, SA, Australia.

Alpha-mannosidosis is a lysosomal storage disorder resulting from deficient activity of lysosomal alpha-mannosidase. It has been described previously in humans, cattle, and cats, and is characterized in all of these species principally by neuronal storage leading to progressive mental deterioration. Two guinea pigs with stunted growth, progressive mental dullness, behavioral abnormalities, and abnormal posture and gait, showed a deficiency of acidic alpha-mannosidase activity in leukocytes, plasma, fibroblasts, and whole liver extracts. Fractionation of liver demonstrated a deficiency of lysosomal (acidic) alpha-mannosidase activity. Thin layer chromatography of urine and tissue extracts confirmed the diagnosis by demonstrating a pattern of excreted and stored oligosaccharides almost identical to that of urine from a human alpha-mannosidosis patient. Widespread neuronal vacuolation was observed throughout the CNS, including the cerebral cortex, hippocampus, thalamus, cerebellum, midbrain, pons, medulla, and the dorsal and ventral horns of the spinal cord. Lysosomal vacuolation also occurred in many other visceral tissues and was particularly severe in pancreas, thyroid, epididymis, and peripheral ganglion. Axonal spheroids were observed in some brain regions, but gliosis and demyelination were not observed. Ultrastructurally, most vacuoles in both the CNS and visceral tissues were lucent or contained fine fibrillar or flocculent material. Rare large neurons in the cerebral cortex contained fine membranous structures. Skeletal abnormalities were very mild. Alpha-mannosidosis in the guinea pig closely resembles the human disease and will provide a convenient model for investigation of new therapeutic strategies for neuronal storage diseases, such as enzyme replacement and gene replacement therapies.

PMID: 10541310 [PubMed - indexed for MEDLINE]

☐ **14:** Biochim Biophys Acta. 1999 Oct 8;1455(2-3):69-84. Related Articles, Links

Glycoprotein lysosomal storage disorders: alpha- and beta-mannosidosis, fucosidosis and alpha-N-acetylgalactosaminidase deficiency.

Michalski JC, Klein A.

Laboratoire de Chimie Biologique, UMR 8576 CNRS (UMR 111 CNRS), Universite des Sciences et Technologies de Lille, Villeneuve d'Ascq, France. jean-claude.michalski@univ-lille1.fr

Glycoproteinoses belong to the lysosomal storage disorders group. The common feature of these diseases is the deficiency of a lysosomal protein that is part of glycan catabolism. Most of the lysosomal enzymes involved in the hydrolysis of glycoprotein carbohydrate chains are exo-glycosidases, which stepwise remove terminal monosaccharides. Thus, the deficiency of a single enzyme causes the blockage of the entire pathway and induces a storage of incompletely degraded substances inside the lysosome. Different mutations may be observed in a single disease and in all cases account for the nonexpression of lysosomal glycosidase activity. Different clinical phenotypes generally characterize a specific disorder,

which rather must be described as a continuum in severity, suggesting that other biochemical or environmental factors influence the course of the disease. This review provides details on clinical features, genotype-phenotype correlations, enzymology and biochemical storage of four human glycoprotein lysosomal storage disorders, respectively alpha- and beta-mannosidosis, fucosidosis and alpha-N-acetylgalactosaminidase deficiency. Moreover, several animal disorders of glycoprotein metabolism have been found and constitute valuable models for the understanding of their human counterparts.

Publication Types:
- Review
- Review, Tutorial

PMID: 10571005 [PubMed - indexed for MEDLINE]

☐ **15:** Exp Mol Pathol. 1999 Jun;66(2):123-30. Related Articles, Links

Comment in:
- Exp Mol Pathol. 2001 Apr;70(2):173-4.

ELSEVIER SCIENCE
FULL-TEXT ARTICLE

Molecular characterization of a novel endonuclease (Xib) and possible involvement in lysosomal glycogen storage disorders.

Malferrari G, Mazza U, Tresoldi C, Rovida E, Nissim M, Mirabella M, Servidei S, Biunno I.

Istituto Tecnologie Biomediche Avanzate-Consiglio Nazionale delle Ricerche, Via Fratelli Cervi 93, Segrate Milano, 20090, Italy.

We cloned and partially characterized a human endonuclease (Xib) which shows sequence homologies to pancreatic DNase I but an enzymatic activity closer to DNase II. We report on the structural differences found between Xib and other recently cloned human DNases. Fluores cence microscopy analysis of transiently transfected cells with Xib::pEGFP constructs indicate that the protein is located in the cytoplasm and possibly anchored to a membrane, as deduced from a hydrophobic amino acid stretch present at the C-terminal end. Xib is overexpressed in muscle and cardiac tissues and is alternately spliced in several normal and neoplastic cells. In situ hybridization studies using human cardiac and muscle biopsies indicate accumulation of Xib transcript in the vacuoles of muscle cells from patients affected by vacuolar myopathy as acid maltase deficiency; however, no point mutations were detected in their DNA. Copyright 1999 Academic Press.

PMID: 10409440 [PubMed - indexed for MEDLINE]

☐ **16:** Neurochem Res. 1999 Apr;24(4):601-15. Related Articles, Links

Gene transfer approaches to the lysosomal storage disorders.

Barranger JA, Rice EO, Swaney WP.

Human Genetics Department at the University of Pittsburgh, PA 15261, USA.

The work summarized in this paper used animal and cell culture models systems to develop gene therapy approaches for the lysosomal storage disorders. The results have provided the scientific basis for a clinical trial of gene transfer to hematopoietic stem cells (HSC) in Gaucher disease which is now in progress. The clinical experiment is providing evidence of HSC transduction, competitive engraftment of genetically corrected HSC, expression of the GC transgene, and the suggestion of a clinical response. In this paper we will review the progress made in Gaucher disease and include how gene transfer might be studied in other lysosomal storage disorders.

Publication Types:
- Review
- Review, Tutorial

PMID: 10227692 [PubMed - indexed for MEDLINE]

☐ **17:** JAMA. 1999 Jan 20;281(3):249-54. Related Articles, Links

JAMA

Prevalence of lysosomal storage disorders.

Meikle PJ, Hopwood JJ, Clague AE, Carey WF.

Department of Chemical Pathology, Women's and Children's Hospital, Adelaide, Australia. p.meikle@medicine.adelaide.edu.au

CONTEXT: Lysosomal storage disorders represent a group of at least 41 genetically distinct, biochemically related, inherited diseases. Individually, these disorders are considered rare, although high prevalence values have been reported in some populations. These disorders are devastating for individuals and their families and result in considerable use of resources from health care systems; however, the magnitude of the problem is not well defined. To date, no comprehensive study has been performed on the prevalence of these disorders as a group. OBJECTIVE: To determine the prevalence of lysosomal storage disorders individually and as a group in the Australian population. DESIGN: Retrospective case studies. SETTING: Australia, from January 1, 1980, through December 31, 1996. MAIN OUTCOME MEASURE: Enzymatic diagnosis of a lysosomal storage disorder. RESULTS: Twenty-seven different lysosomal storage disorders were diagnosed in 545 individuals. The prevalence ranged from 1 per 57000 live births for Gaucher disease to 1 per 4.2 million live births for sialidosis. Eighteen of 27 disorders had more than 10 diagnosed cases. As a group of disorders, the combined prevalence was 1 per 7700 live births. There was no significant increase in the rate of either clinical diagnoses or prenatal diagnoses of lysosomal storage disorders during the study period. CONCLUSIONS: Individually, lysosomal storage disorders are rare genetic diseases. However, as a group, they are relatively common and represent an important health problem in Australia.

PMID: 9918480 [PubMed - indexed for MEDLINE]

☐ **18:** FEBS Lett. 1998 Dec 28;441(3):369-72. Related Articles, Links

ELSEVIER SCIENCE
FULL-TEXT ARTICLE

Application of magnetic chromatography to the isolation of lysosomes from fibroblasts of patients with lysosomal storage disorders.

Diettrich O, Mills K, Johnson AW, Hasilik A, Winchester BG.

Institute of Child Health, London, UK. o.diettrich@ich.ucl.ac.uk

A method for the purification of lysosomes from fibroblasts has been developed which uses endocytosis of superparamagnetic colloidal iron dextran particles followed by separation of the iron-containing lysosomes in a magnetic field. This permitted isolation of lysosomes from fibroblasts from patients with infantile sialic acid storage disorder and other lysosomal storage diseases in which a shift in lysosomal density induced by the storage material prevents purification by centrifugation in a Percoll gradient. The magnetic lysosomes isolated from these cells are very similar to those from normal cells as judged by lysosomal marker enzyme activity and 2D-PAGE analysis of the enriched proteins.

PMID: 9891973 [PubMed - indexed for MEDLINE]

☐ **19:** Clin Chem. 1998 Oct;44(10):2094-102. Related Articles, Links

FREE full text article at
www.clinchem.org

Evaluation of the lysosome-associated membrane protein LAMP-2 as a marker for lysosomal storage disorders.

Hua CT, Hopwood JJ, Carlsson SR, Harris RJ, Meikle PJ.

Department of Chemical Pathology, Women's and Children's Hospital, North Adelaide, South Australia, Australia.

For many lysosomal storage disorders, presymptomatic detection, before the onset of irreversible pathology, will greatly improve the efficacy of current and proposed therapies. In the absence of a family history, presymptomatic detection can be achieved only by a comprehensive newborn screening program. Recently we reported that the lysosome-associated membrane protein LAMP-1 was increased in the plasma from approximately 70% of individuals with lysosomal storage disorders. Here we report on the evaluation of a second lysosome-associated membrane protein, LAMP-2, as a marker for this group of disorders. The median concentration of LAMP-2 in the plasma of healthy individuals was 1.21 mg/L, fourfold higher than the median LAMP-1 concentration (0.31 mg/L). LAMP-2 was increased in >66% of patients with lysosomal storage disorders, and the increases coincided with increased LAMP-1 concentrations. The reference intervals for LAMP-1 and LAMP-2 in blood spots taken from newborns were 0.20-0.54 mg/L (n = 1600) and 0.95-3.06 mg/L (n = 1600), respectively. A high correlation was observed between the concentrations of LAMP-1 and LAMP-2 in both control and affected individuals. The higher concentrations of LAMP-2, relative to LAMP-1, in plasma make LAMP-2 an attractive marker; however, the final selection will be dependent on the availability of new diagnostic markers and their ability to detect disorders currently not identified by LAMP-2.

PMID: 9761240 [PubMed - indexed for MEDLINE]

20: Ryoikibetsu Shokogun Shirizu. 1998;(19 Pt 2):601-5. Related Articles, Links

[Lysosomal transport disorders: cystinosis and sialic acid storage disorders]

[Article in Japanese]

Eto Y.

Department of Pediatrics, Tokyo Jikei University School of Medicine.

Publication Types:
- Review
- Review, Tutorial

PMID: 9645145 [PubMed - indexed for MEDLINE]

21: Brain Pathol. 1998 Jan;8(1):175-93. Related Articles, Links

Cellular pathology of lysosomal storage disorders.

Walkley SU.

Department of Neuroscience, Rose F. Kennedy Center for Research in Mental Retardation and Human Development, Albert Einstein College of Medicine, Bronx, NY 10461, USA. walkley@aecom.yu.edu

Lysosomal storage disorders are rare, inborn errors of metabolism characterized by intralysosomal accumulation of unmetabolized compounds. The brain is commonly a central focus of the disease process and children and animals affected by these disorders often exhibit progressively severe neurological abnormalities. Although most storage diseases result from loss of activity of a single enzyme responsible for a single catabolic step in a single organelle, the lysosome, the overall features of the resulting disease belies this simple beginning. These are enormously complex disorders with metabolic and functional consequences that go far beyond the lysosome and impact both soma-dendritic and axonal domains of neurons in highly neuron type-specific ways. Cellular pathological changes include growth of ectopic dendrites and new synaptic connections and formation of enlargements in axons far distant from the lysosomal defect. Other storage diseases exhibit neuron death, also occurring in a cell-selective manner. The functional links between known molecular genetic and enzyme defects and changes in neuronal integrity remain largely unknown. Future studies on the biology of lysosomal storage diseases affecting the brain can be anticipated to provide insights not only into these pathogenic mechanisms, but also into the role of lysosomes and related organelles in normal neuron function.

Publication Types:
- Review
- Review, Tutorial

PMID: 9458175 [PubMed - indexed for MEDLINE]

22: Clin Chem. 1997 Aug;43(8 Pt 1):1325-35. Related Articles, Links

FREE full text article at www.clinchem.org

Diagnosis of lysosomal storage disorders: evaluation of lysosome-associated membrane protein LAMP-1 as a diagnostic marker.

Meikle PJ, Brooks DA, Ravenscroft EM, Yan M, Williams RE, Jaunzems AE, Chataway TK, Karageorgos LE, Davey RC, Boulter CD, Carlsson SR, Hopwood JJ.

Department of Chemical Pathology, Women's and Children's Hospital, North Adelaide, Australia. omeikle@medicine.adelaide.edu.au

Early diagnosis of lysosomal storage disorders (LSDs), before the onset of irreversible pathologies, will be a key factor in the development of effective therapies for many of these disorders. Newborn screening offers a potential mechanism for the early detection of these disorders. From studies of both normal and LSD-affected human skin fibroblasts we identified the lysosome-associated membrane protein LAMP-1 as a potential diagnostic marker. We have developed a sensitive method for the quantification of this protein with a time-resolved fluorescence immunoassay. A soluble form of LAMP-1 was observed in plasma samples, and determination of 152 unaffected individuals gave a median value of 303 micrograms/L with the 5th and 95th percentile at 175 and 448 micrograms/L respectively. Plasma samples from 320 LSD-affected individuals representing 25 different disorders were assayed. We observed that 17 of the 25 disorder groups tested had > 88% of individuals above the 95th percentile of the control population, with 12 groups having 100% above the 95th percentile. Overall, 72% of patients had LAMP-1 concentrations above the 95th percentile of the unpartitioned control population. We suggest that LAMP-1 may be a useful marker in newborn screening for LSDs.

PMID: 9267309 [PubMed - indexed for MEDLINE]

23: Exp Cell Res. 1997 Jul 10;234(1):85-97. Related Articles, Links

ELSEVIER SCIENCE FULL-TEXT ARTICLE

Lysosomal biogenesis in lysosomal storage disorders.

Karageorgos LE, Isaac EL, Brooks DA, Ravenscroft EM, Davey R, Hopwood JJ, Meikle PJ.

Department of Chemical Pathology, Women's and Children's Hospital, North Adelaide, South Australia.

Lysosomal biogenesis is an orchestration of the structural and functional elements of the lysosome to form an integrated organelle and involves the synthesis, targeting, functional residence, and turnover of the proteins that comprise the lysosome. We have investigated lysosomal biogenesis during the formation and dissipation of storage vacuoles in two model systems. One involves the formation of sucrosomes in normal skin fibroblasts and the other utilizes storage disorder-affected skin fibroblasts; both of these systems result in an increase in the size and the number of lysosomal vacuoles. Lysosomal proteins, beta-hexosaminidase, alpha-mannosidase, N-acetylgalactosamine-4-sulfatase, acid phosphatase,

and the lysosome-associated membrane protein, LAMP-1, were shown to be elevated between 2- and 28-fold above normal during lysosomal storage. Levels of mRNA for the lysosome-associated membrane proteins LAMP-1 and LAMP-2, N-acetylgalactosamine-4-sulfatase, and the 46- and 300-kDa mannose-6-phosphate receptors were also elevated 2- to 8-fold. The up-regulation of protein and mRNA lagged 2-4 days behind the formation of lysosomal storage vacuoles. Correction of storage, in both systems, resulted in the rapid decline of the mRNA to basal levels, with a slower decrease in the levels of lysosomal proteins. Lysosomal biogenesis in storage disorders is shown to be a regulated process which is partially controlled at, or prior to, the level of mRNA. Although lysosomal proteins were differentially regulated, the coordination of these events in lysosomal biogenesis would suggest that a common mechanism(s) may be in operation.

PMID: 9223373 [PubMed - indexed for MEDLINE]

☐ **24:** Nippon Rinsho. 1995 Dec;53(12):3068-71. Related Articles, Links

[Lysosomal membrane transport disorders--cystinosis and sialic acid storage disorders (Salla disease, ISSD)]

[Article in Japanese]

Yano T, Ohno K.

Department of Neurobiology School of Life Science, Tottori University, Faculty of Medicine.

Cystinosis and sialic acid storage diseases (Salla disease, ISSD; infantile sialic acid storage disease) are lysosomal membrane disorders resulting from defective carrier-mediated transport of cystine and sialic acid across the lysosomal membrane. Both are rare autosomal recessively inherited disorders. The major clinical manifestations of cystinosis are renal failure and ocular damages. Sialic acid storage diseases are characterized by various degrees of psychomotor retardation. Salla disease patients trace a mild clinical course, and the life span is relatively long. While, in patients with ISSD follow a very severe progressive clinical course and often die in the first year of life. The genes responsible for each disease have not been isolated, the etiologies are not well known, and there is no specific treatment.

Publication Types:
- Review
- Review, Tutorial

PMID: 8577060 [PubMed - indexed for MEDLINE]

☐ **25:** Pediatr Pol. 1995 Oct;70(10):847-55. Related Articles, Links

[Thin-layer chromatography of urine oligosaccharides in diagnosis of some lysosomal storage disorders]

[Article in Polish]

Lugowska A, Tylki-Szymanska A, Sawnor-Korszynska D.

Zaklad Diagnostyki Laboratoryjnej Centrum Zdrowia Dziecka w Warszawie.

Inherited lysosomal storage disorders are caused by the deficiency or importantly lowered activity of one of the lysosomal enzymes, leading to the storage in the lysosomes the not degraded high-molecular substrates, among others: mucopolysaccharides, glycolipids, oligosaccharides and glycoproteins. Thin-layer chromatography of urine oligosaccharides allows reliable and fast diagnosis of some lysosomal storage disorders e.g. alpha-mannosidosis, fucosidosis, sialidosis, galactosialidosis, Schindler disease, GM1-gangliosidosis, GM2-gangliosidosis (Sandhoff type), Pompe disease, Salla disease, mucolipidosis II and III. We are presenting a modification of the Humbel and Collart's method of TLC of urine oligosaccharides. The principle of our modification is to introduce of the preliminary desalting step of the urine on the columns containing anionit BioRad AG 1 x 8 and cationit Dowex 50 x 8-200.

PMID: 8649932 [PubMed - indexed for MEDLINE]

26: Am J Hum Genet. 1995 Oct;57(4):893-901. Related Articles, Links

Lysosomal free sialic acid storage disorders with different phenotypic presentations--infantile-form sialic acid storage disease and Salla disease--represent allelic disorders on 6q14-15.

Schleutker J, Leppanen P, Mansson JE, Erikson A, Weissenbach J, Peltonen L, Aula P.

Department of Medical Genetics, University of Turku, Finland.

Similarities in biochemical findings have suggested that Salla disease (SD) and the infantile form of sialic acid storage disease (ISSD) could represent allelic disorders, despite their drastically different clinical phenotypes. SD and ISSD are both characterized by lysosomal storage of free N-acetyl neuraminic acid. However, in SD the increase detected in urine is 8-24-fold, whereas in ISSD the corresponding amount is 20-50-fold and patients are also more severely affected. Here we report linkage studies in 50 Finnish SD families and 26 non-Finnish families with no genealogical connections to Finns affected either with the Finnish type of SD, the "intermediate" form of the disease, or ISSD. All forms of the disease show linkage to the same locus on 6q14-q15. Haplotype analyses of Finnish SD chromosomes revealed one common haplotype, which was also seen in most of the non-Finnish patients with Finnish type of SD. This ancestral haplotype deviated from those observed in ISSD patients, who had a different common haplotype.

PMID: 7573051 [PubMed - indexed for MEDLINE]

27: J Inherit Metab Dis. 1995;18(6):717-22. Related Articles, Links

Elevated plasma chitotriosidase activity in various lysosomal storage disorders.

Guo Y, He W, Boer AM, Wevers RA, de Bruijn AM, Groener JE, Hollak CE, Aerts JM, Galjaard H, van Diggelen OP.

Department of Clinical Genetics, Erasmus University, Rotterdam, The Netherlands.

Recently a striking elevation of the activity of chitotriosidase, an endo beta-glucosaminidase distinct from lysozyme, was found in plasma from patients with Gaucher type I disease (McKusick 230800). Plasma chitotriosidase originates from activated macrophages and this elevation is secondary to the basic defect in Gaucher disease. To investigate the specificity of this phenomenon, we have investigated 24 different lysosomal storage diseases. In 11 different diseases increased chitotriosidase activity in plasma was found (in 28% of the patients). None of these diseases showed elevations as high as in Gaucher disease. Chitotriosidase was not significantly elevated in plasma from 20 different non-lysosomal enzymopathies or in plasma from patients with infectious diseases associated with hepatomegaly. The results show that marked elevation of chitotriosidase activity in plasma appears to be specific for Gaucher disease. The data further suggest that elevated levels of chitotriosidase activity in plasma from patients with unexplained diseases may be indicative for a lysosomal disorder.

PMID: 8750610 [PubMed - indexed for MEDLINE]

28: Southeast Asian J Trop Med Public Health. 1995;26 Suppl 1:54-8. Related Articles, Links

Lysosomal storage disorders in Thailand: the Siriraj experience.

Wasant P, Wattanaweeradej S, Raksadawan N, Kolodny EH.

Department of Pediatrics, Siriraj Hospital Medical School, Mahidol University, Bangkok, Thailand.

Lysosomal storage disorders are a heterogeneous group of biochemical genetic disorders; currently 40-50 are known. The clinical phenotype is determined by the tissue distribution of the storage material and degree of enzyme deficiency. The genetic transmission is mostly autosomal recessive. Lysosomal storage disorders can be divided into three groups according to the major organ system pathology: (1) Primary involvement of the central nervous system without significant somatic or skeletal pathology. Disorders of grey matter, eg gangliosidosis and disorders of white matter eg the leucodystrophy are the most common; (2) Primary involvement of the reticuloendothelial system with or without associated neuropathology, eg Niemann-Pick disease and Gaucher disease; (3) Multisystem involvement in which skeletal manifestations are prominent features. The mucopolysaccharidosis and mucolipidoses are the two major forms with this clinical phenotype. Lysosomal storage disorders identified at Siriraj Hospital are neuronal ceroid lipofuscinosis, GMI gangliosidosis, mucolipidosis II, Maroteaux-Lamy, sialidosis, Sly syndrome, Hunter syndrome, Morquio syndrome, Gaucher disease, Niemann-Pick, Sandhoff disease, Pompe's disease and many more. Most patients came from the provinces where consanguinity is common. Confirmation usually is done by enzyme assays using skin fibroblast culture or leucocytes. Genetic counseling is extremely important and prenatal diagnosis is recommended to high-risk couple.

Publication Types:
- Case Reports

PMID: 8629143 [PubMed - indexed for MEDLINE]

29: Br Med Bull. 1995 Jan;51(1):106-22. Related Articles, Links

Gene therapy of lysosomal storage disorders.

Salvetti A, Heard JM, Danos O.

Retrovirus et Transfert Genetique, Institut Pasteur, Paris, France.

Lysosomal storage disorders (LSD) result from deficiencies in enzymes normally implicated in the catabolism of macromolecules inside the lysosome. Many of these enzymes can reach the lysosome after being secreted in the extracellular medium and recaptured by specific cell surface receptors. This has suggested a rationale for therapeutic approaches in LSD, in which the missing enzyme is provided by an external source. Current therapies based on this concept, including the administration of purified enzyme and bone marrow transplantation, have been shown to result in clinical improvements in both animal models and patients. Although considerable difficulties must be surmounted, LSD present a favourable situation for gene therapy. The gene corresponding to the affected enzyme has been identified in most diseases and cDNAs are available. Low and unregulated levels of enzyme activity should be sufficient for correction. Importantly, a variety of gene transfer strategies can be carefully evaluated in animal models.

Publication Types:
- Review
- Review, Academic

PMID: 7767637 [PubMed - indexed for MEDLINE]

30: Biochim Biophys Acta. 1994 May 25;1226(2):138-44. Related Articles, Links

Free sphingoid bases in tissues from patients with type C Niemann-Pick disease and other lysosomal storage disorders.

Rodriguez-Lafrasse C, Rousson R, Pentchev PG, Louisot P, Vanier MT.

Department of Biochemistry, INSERM-CNRS 189, Lyon-Sud Medical School, Oullins, France.

The 20-fold increase of free sphingoid bases found in liver from a murine model of Niemann-Pick type C (NPC) combined to the NPC-like phenotype induced by addition of sphinganine to normal fibroblast cultures prompted us to investigate the potential involvement of these compounds in the human disease. The contents of sphingosine and sphinganine were measured in liver, spleen, brain and skin fibroblast cultures by a sensitive HPLC method. In liver and spleen from NPC patients, a 6- to 24-fold elevation of sphingosine and sphinganine already prominent at the fetal stage of the disease was observed, while no clear increase could be evidenced in brain tissue. A significant increase, not modulated by the intralysosomal content of free cholesterol, also occurred in skin

fibroblast cultures. To investigate the specificity of these findings, other lysosomal storage disorders were studied. A striking accumulation was found in liver and spleen (24- to 36-fold) from patients with Niemann-Pick disease type A and B (sphingomyelinase-deficient forms), and in cerebral cortex of type A Niemann-Pick disease. A significant storage also occurred in Sandhoff disease, while several other sphingolipidoses showed a moderate elevation. In all cases but Sandhoff disease brain, the sphingosine/sphinganine ratio remained unchanged, suggesting that the accumulated free sphingoid bases derived from sphingolipid catabolism. Formation of complexes between sphingosine and the lipid material accumulated in lysosomes might be a general mechanism in lysosomal lipidoses. In NPC, however, an increase of free sphingoid bases disproportionate to the degree of lysosomal storage and a specific involvement of cultured fibroblasts suggested a more complex or combined mechanism.

PMID: 8204660 [PubMed - indexed for MEDLINE]

31: Biol Cell. 1994;81(2):143-52. Related Articles, Links

Effects of suramin, a polyanionic drug inducing lysosomal storage disorders on tooth germs in vitro.

Gritli-Linde A, Ruch JV, Mark MP, Lecolle S, Goldberg M.

Laboratoire de Biologie et Biomateriaux du Milieu Buccal et Osseux, Faculte de Chirurgie Dentaire LA 1505, Montrouge, France.

Suramin, a potent inhibitor of lysosomal enzymes, is commonly employed as a tool for inducing experimental mucopolysaccharidosis and lipidosis. The effects of the drug on embryonic mouse molars were analysed. Presecretory ameloblasts and odontoblasts were loaded with lysosome-like vacuoles. Staining with MC22-33F, an antibody to choline phospholipids and sphingomyelin, was completely reversed in the suramin-treated germs, in that it stained only presecretory ameloblasts (versus odontoblasts and some pulpal cells in the control group), according to a developmentally regulated pattern. The suramin-induced cytoplasmic changes were reminiscent of the features of mucopolysaccharidoses and lipidoses. The basement membrane, separating the enamel organ from the dental papilla, displayed suramin-induced patches, and in predentin collagen fibrillogenesis was found to be disturbed. Furthermore, autoradiography was employed to reveal uptake and distribution of [3H] suramin in the cells and predentin. Finally, a suramin-induced disturbance of the metabolism of sulphated macromolecules was found. The results imply that suramin effects in vitro on tooth germs can be used as a useful experimental model with to study both the action of the drug as well as cell and extracellular matrix perturbations in a mucopolysaccharidosis-like condition.

PMID: 7849606 [PubMed - indexed for MEDLINE]

32: Nippon Rinsho. 1993 Sep;51(9):2264-8. Related Articles, Links

[Lysosomal storage disease: a group of genetic neurodegenerative disorders]

[Article in Japanese]

Suzuki Y.

Tokyo Metropolitan Institute of Medical Science.

Lysosomal storage disease is a group of neurometabolic diseases mainly occurring in infancy and childhood. They were first recognized as new diseases on the basis of unique clinical manifestations or pathological findings, and then the stage of biochemical analysis of storage material and enzyme assays in tissues and cells from patients followed. Recent technological development has enabled us to look further into the molecular genetic basis of these inherited diseases. Protein analysis revealed intracellular events of the mutant enzyme molecule responsible for the pathogenesis of a disease, and more detailed information has been obtained about the mutant gene and its product. Clinical manifestations are not always uniform for a single disease with mutations in the same gene. Clinical subtypes have been proposed for many lysosomal diseases. At present, the molecular and metabolic basis of each phenotypic expression is not clear, although common mutations have been found for specific clinical forms in some diseases. In this article, the current status of lysosomal disease research was summarized, particularly focusing on molecular pathology and molecular diagnosis. Finally future prospects for pathogenetic analysis of neural dysfunction and possible gene therapy were briefly discussed.

Publication Types:
- Review
- Review Literature

PMID: 8411700 [PubMed - indexed for MEDLINE]

33: J Inherit Metab Dis. 1993;16(2):288-91. Related Articles, Links

Pathogenesis of lysosomal storage disorders as illustrated by Gaucher disease.

Aerts JM, Van Weely S, Boot R, Hollak CE, Tager JM.

E.C. Slater Institute for Biochemical Research, University of Amsterdam, The Netherlands.

Publication Types:
- Review
- Review, Tutorial

PMID: 8411983 [PubMed - indexed for MEDLINE]

34: C R Seances Soc Biol Fil. 1993;187(5):596-607. Related Articles, Links

[Lysosomal storage diseases, genetic or drug-induced? effect of glycosaminoglycan and sphingolipid disorders on dental tissues]

[Article in French]

Goldberg M, Gritli A, Bloch-Zupan A, Septier D, Lecolle S, Legrand JM, Ruch JV.

Laboratoire de Biologie et Biomateriaux du Milieu Buccal et Osseux, Faculte de Chirurgie Dentaire, Paris V, Montrouge, France.

In vivo studies were carried out on dental tissues of rat incisor after a single injection of suramin, a drug which induces mucopolysaccharidosis-like disease. Accumulation of lysosome-like structures was seen in secretory ameloblasts and odontoblasts. In vitro studies on embryonic tooth germ buds showed similar changes when they were cultured in presence of suramin. Anti-phospholipid immunolabelling revealed a developmentally regulated temporo-spatial pattern. Radiolabeling with 3H-suramin indicated cytosolic and nuclear incorporation. The drug acting as polyanion interacted directly with predentine. 35S sulphate incorporation was impaired by the drug. Another lysosomal storage disease, the sphingolipidosis, Krabbe's disease was also investigated in human. Changes were observed in pulp cells and as a consequence in dentin. Enamel also displayed many changes. Pharmacological or genetically acquired diseases constitute models providing insights on the role played by glycosaminoglycans and phospholipids in biomineralization.

PMID: 8069712 [PubMed - indexed for MEDLINE]

☐ **35:** Clin Neuropathol. 1992 Sep-Oct;11(5):251-5. Related Articles, Links

Neuronal ubiquitin and neurofilament expression in different lysosomal storage disorders.

Zhan SS, Beyreuther K, Schmitt HP.

Institute of Neuropathology, University of Heidelberg, Germany.

We studied various lysosomal storage disorders such as Tay-Sachs' disease, Niemann-Pick's disease, and Hunter's disease for their immunoreactivity with antibodies against ubiquitin (Ub) and neurofilaments (NF). We found that in all cases, irrespective of the nature of the storage material or disorder, only a minor proportion of neurons (20-30% at most), as a rule, moderately reacted with the Ub antibody, while the majority of the distended neurons neither expressed Ub nor NF epitopes. These findings suggest that the UB dependent proteolytic pathway may play a secondary role in the lysosomal storage disorders, at least in the advanced stages which are observed at autopsy. It seems that the Ub expression of a minor proportion of neurons should be regarded as an unspecific epiphenomenon rather than as a mechanism of major significance in the basic metabolism of these disorders, in which the inclusions consist of membrane-bound lipid material.

PMID: 1385029 [PubMed - indexed for MEDLINE]

☐ **36:** FASEB J. 1991 Mar 1;5(3):301-8. Related Articles, Links

Saposin proteins: structure, function, and role in human lysosomal storage disorders.

O'Brien JS, Kishimoto Y.

Department of Neurosciences, University of California, San Diego, La Jolla 92093.

Saposins are sphingolipid activator proteins, four of which are derived from a single precursor, prosaposin, by proteolytic processing. These small heat-stable glycoproteins (12-14 kDa) are required for the lysosomal hydrolysis of a variety of sphingolipids. Characterization of these four activator proteins, two of which were recently discovered, and their importance in human health and disease are reviewed in this article.

Publication Types:
- Review
- Review, Academic

PMID: 2001789 [PubMed - indexed for MEDLINE]

☐ **37:** In Vitro Cell Dev Biol. 1988 Dec;24(12):1159-64. Related Articles, Links

Culture conditions found to minimize false positive diagnosis of lysosomal storage disorders.

Arnon J, Ornoy A, Bach G.

Dept. of Anatomy and Embryology, Hadassah Medical School; Jerusalem, Israel.

The effect of culture conditions on the ultrastructure and enzyme activities of cultured skin fibroblast cells relevant to the diagnosis of lysosomal storage disorders are reported. The parameters examined were: pH of the culture media, type of media, increasing cell passage, and day of harvest. Ultrastructural changes were defined in terms of the number of lysosome-like inclusion bodies per cell according to a method devised in our laboratory and proven reliable in the detection of affected individuals. Our biochemical results included determination of enzyme activities of beta-hexosaminidase, alpha-mannosidase, beta-glucuronidase-lysosomal enzymes, arylsulfatase C, a microsomal marker, and 5' nucleotidase, a plasma membrane marker. Our results indicate that the cellular ultrastructure is more sensitive than enzyme activity to changes in culture conditions. The resulting ultrastructural "artifacts" observed under certain conditions were severe enough to result in a mistaken diagnosis. Due to certain difficulties we had previously encountered in heterozygote cultures (for lysosomal storage disorders) of amniotic cells, we decided to examine heterozygote cultures of skin fibroblasts. From these (preliminary) studies it seems that an elevation in the pH over the physiologic levels in the culture media may help to define between normal individuals and affected heterozygotes. On the basis of our results, we recommend that to minimize false positive ultrastructural results for the diagnosis of lysosomal storage disorders, cultures be grown in minimal essential medium, the pH of the medium carefully monitored to remain below 7.4, examining the cultures not later than cell Passage 8 and no later than Day 10 after subculture.

PMID: 3209585 [PubMed - indexed for MEDLINE]

☐ **38:** Am J Hum Genet. 1988 Feb;42(2):271-3. Related Articles, Links

Selection in favor of lysosomal storage disorders?

Zlotogora J, Zeigler M, Bach G.

Department of Human Genetics, Hadassah University Hospital, Jerusalem, Israel.

Four examples of Israeli communities or large families in which high consanguinity is common are presented, with two different lysosomal storage disorders within each community. In each of the four cases the stored substances share common chemical structure, despite the different lysosomal hydrolases involved in each disease. A similar phenomenon is known among the Ashkenazi Jews, in whom four of the most frequent hereditary disorders are lysosomal storage disorders, which are characterized by storage of sphingolipid derivatives. Similar findings are reported in the literature in other communities. We suggest that this phenomenon indicates a selection in favor of lysosomal storage disorders of similar nature in certain populations. The selection forces leading to this phenomenon have not been identified yet, and it has not yet been determined whether these forces are the same in the different communities presented here.

Publication Types:
- Case Reports

PMID: 3124612 [PubMed - indexed for MEDLINE]

☐ **39:** Trans Am Ophthalmol Soc. 1987;85:471-97. Related Articles, Links

The efficacy of conjunctival biopsy as a screening technique in lysosomal storage disorders.

Mazow ML.

Publication Types:
- Review
- Review of Reported Cases

PMID: 3328919 [PubMed - indexed for MEDLINE]

☐ **40:** Prenat Diagn. 1986 Sep-Oct;6(5):351-61. Related Articles, Links

Cultured amniotic fluid cells for prenatal diagnosis of lysosomal storage disorders: a methodological study.

Arnon J, Ornoy A, Bach G.

The influence of culture conditions on the ultrastructure and enzyme activities of amniotic fluid cells are reported. Morphological changes were determined as a function of the number of lysosomal-like inclusion bodies per cell, and these results correlated to the activity of beta-hexosaminidase, alpha-mannosidase, beta-glucuronidase, arylsulphatase C and 5' nucleotidase. The parameters examined were pH of the culture media, type of media, increasing cell passage and day of harvest. Our results indicate that enzyme activities are

less sensitive to changes in culture conditions as compared to ultrastructural changes. We therefore recommend that in order to obtain reliable ultrastructural results for the diagnosis of storage disorders, cultures should be grown in MEM as the culture medium, the pH of the medium carefully monitored to remain below pH 7.4, examining the cultures no later than the eighth cell passage and no later than the 10th day after subculture.

PMID: 3022278 [PubMed - indexed for MEDLINE]

41: Hum Genet. 1986 Jul;73(3):214-7. Related Articles, Links

Free N-acetylneuraminic acid (NANA) storage disorders: evidence for defective NANA transport across the lysosomal membrane.

Mancini GM, Verheijen FW, Galjaard H.

To study the biochemical defect underlying N-acetylneuraminic acid (NANA) storage disorders (NSD), a tritium-labeled NANA-methylester was prepared and its metabolism was studied in normal and mutant human fibroblasts. The uptake of methylester, its conversion into free NANA, and the release of free NANA was studied in lysosome-enriched fractions. In three clinically different types of NSD accumulation of free NANA was observed and the half-life of this compound was significantly increased. Our observations indicate the existence of a transport system for NANA across the lysosomal membrane, which is deficient in all variants of NSD.

PMID: 3733077 [PubMed - indexed for MEDLINE]

42: Lancet. 1985 Dec 7;2(8467):1296. Related Articles, Links

Pseudodeficiencies in lysosomal storage disorders.

Zlotogora J, Bach G.

Publication Types:
- Letter

PMID: 2866352 [PubMed - indexed for MEDLINE]

43: Med J Aust. 1984 Feb 18;140(4):188-9. Related Articles, Links

Diagnosis of lysosomal storage disorders.

Kerr C.

PMID: 6694619 [PubMed - indexed for MEDLINE]

44: Virchows Arch B Cell Pathol Incl Mol Pathol. 1984;46(1-2):13-9. Related Articles, Links

Ito cells in lysosomal storage disorders. An ultrastructural study.

Elleder M.

An ultrastructural study was performed in a series of liver biopsies from patients with various lysosomal storage diseases to evaluate the extent of lysosomal hypertrophy and hyperplasia in Ito cells (ICs). In previous studies this has been considered to be absent or only rudimentary. Lysosomal storage was recognized by the presence of storage cytosomes surrounded by limiting membranes and by the appearance of their content which was identical to that in other hepatic storage lysosomes. Storage was found in sphingomyelinase deficiency (Niemann-Pick disease types A, B), in Wolman's disease, GM1 gangliosidosis, mucopolysaccharidosis and in multiple sulphatase deficiency. In type C Niemann-Pick disease it was virtually absent with the exception of cases with prominent hepatic symptomatology. Storage was of variable degree and was accompanied by a decrease in the physiological fat content (cytoplasmic lipid droplets). The degree to which ICs were affected correlated only with the extent to which nonspecific fibroblasts were involved in the specimens studied and thus seems to reflect storage in the fibroblastic population.

PMID: 6147922 [PubMed - indexed for MEDLINE]

45: Anal Biochem. 1980 Feb;102(1):213-9. Related Articles, Links

High-performance liquid chromatographic analysis of oligosaccharides and glycopeptides accumulating in lysosomal storage disorders.

Kin NM, Wolfe LS.

PMID: 6766687 [PubMed - indexed for MEDLINE]

46: Front Biol. 1979;48:49-130. Related Articles, Links

Drug-induced lysosomal storage disorders.

Lullmann-Rauch R.

Publication Types:
- Review

PMID: 387466 [PubMed - indexed for MEDLINE]

47: Birth Defects Orig Artic Ser. 1976;12(3):1-13. Related Articles, Links

Chemical diagnosis of inborn lysosomal storage disorders involving the eye.

Dawson G, Tsay GC.

Publication Types:
- Review

PMID: 821555 [PubMed - indexed for MEDLINE]

48: Arch Neurol. 1975 Sep;32(9):592-9. Related Articles, Links

Lysosomal storage disorders. Diagnosis by ultrastructural examination of skin biopsy specimens.

O'Brien JS, Bernett J, Veath ML, Paa D.

Fifteen patients with lysosomal storage diseases were studied. Diagnoses of their illnesses included infantile Gaucher disease; Krabbe disease; Niemann-Pick disease, type A; glycogen storage disease, type 3; Fabry disease, Jansky-Bielschowsky and Spielmeyer-Vogt types of amaurotic idiocy, GM1 gangliosidosis, type 1; Hurler disease; and Sanfilippo disease, types A and B. We carried out ultrastructural examinations of skin biopsy specimens that were taken to establish a cultured fibroblast line on each patient. We found diagnostic storage inclusions in all patients except those with infantile Gaucher disease, Krabbe disease, and Spielmeyer-Vogt disease, This technique can be carried out on a specimen obtained by a primary physician on an out-patient basis, thus avoiding major surgery.

PMID: 809024 [PubMed - indexed for MEDLINE]

Field: **Title,** Limits: **Publication Date to 2001/10/29**

Display Abstract Show: 50 Sort Send to Text

Items 1-48 of 48

One page.

1: J Inherit Metab Dis. 2001;24 Suppl 2:47-51; discussion 45-6. Related Articles, Links

kluweronline
Full text article

Variable clinical presentation in lysosomal storage disorders.

Beck M.

Children's Hospital, University of Mainz, Germany. Beck@kinder.klinik.uni-mainz.de

Extensive clinical heterogeneity is seen in lysosomal storage disorders, regarding the age of onset and severity of symptoms, the organs involved, and effects on the central nervous system. A broad phenotypic spectrum is seen, for example, in mucopolysaccharidosis type I (Hurler/Scheie disease), Gaucher disease, the several forms of GM2-gangliosidosis and the different manifestations of beta-galactosidase deficiency (GM1-gangliosidosis and Morquio disease type B). Variable clinical expression of the same enzyme defect is not well understood. The presence of different mutations is only part of the explanation, as intrafamilial variability is observed in many cases. Other mechanisms, for example the effect of specific activators, may also have an influence on phenotype.

Publication Types:
- Review
- Review, Tutorial

PMID: 11758678 [PubMed - indexed for MEDLINE]

2: Chem Rev. 2000 Dec 13;100(12):4683-96. Related Articles, Links

ACS PUBLICATIONS

Inhibition of glycosphingolipid biosynthesis: application to lysosomal storage disorders.

Butters TD, Dwek RA, Platt FM.

Oxford Glycobiology Institute, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, U.K.

PMID: 11749362 [PubMed - as supplied by publisher]

3: Expert Opin Biol Ther. 2001 Sep;1(5):857-67. Related Articles, Links

Gene therapy for lysosomal storage disorders.

Barranger JM, Novelli EA.

University of Pittsburgh, Department of Human Genetics, PA, USA.
jbarrang@helix.hgen.pitt.edu

The lysosomal storage disorders (LSD) are monogenic inborn errors of metabolism with heterogeneous pathophysiology and clinical manifestations. In the last decades, these disorders have been models for the development of molecular and cellular therapies for inherited metabolic diseases. Studies in preclinical in vitro systems and animal models have allowed the successful development of bone marrow transplantation (BMT) and enzyme replacement therapy (ERT) as therapeutic options for several LSDs. However, BMT is limited by poor donor availability and high morbidity and mortality, and ERT is not a life-long cure. Moreover, the neuropathology present in many LSDs responded poorly, if at all, to these treatments. Therefore, gene therapy is an attractive therapeutic alternative. Gene therapy strategies for LSDs have employed ex vivo gene transduction of cellular targets with subsequent transplantation of the enzymatically corrected cells, or direct in vivo delivery of the viral vectors. Oncoretroviral vectors and more recently adeno associated vectors (AAV) and lentiviral vectors have been extensively tested, with some success. This review summarises the main gene therapy strategies which have been employed or are under development for both non-neurological and neuronopathic LSDs. Some of the in vitro and in vivo preclinical studies presented herein have provided the rationale for a gene therapy clinical trial for Gaucher disease Type I.

Publication Types:
- Review
- Review, Tutorial

PMID: 11728220 [PubMed - indexed for MEDLINE]

4: Eur J Paediatr Neurol. 2001;5 Suppl A:73-9. Related Articles, Links

Tripeptidyl-peptidase I in neuronal ceroid lipofuscinoses and other lysosomal storage disorders.

Wisniewski KE, Kida E, Walus M, Wujek P, Kaczmarski W, Golabek AA.

Department of Pathological Neurobiology, New York State Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Road, Staten Island, NY 10314, USA.

The classic late infantile form of neuronal ceroid lipofuscinosis (CLN2, cLINCL) is associated with mutations in the gene encoding tripeptidyl-peptidase I (TPP-I), a lysosomal aminopeptidase that cleaves off tripeptides from the free N-termini of oligopeptides. To date over 30 different mutations and 14 polymorphisms associated with CLN2 disease process have been identified. In the present study, we analysed the molecular basis of 15

different mutations of TPP-I by using immunocytochemistry, immunofluorescence, Western blotting, enzymatic assay and subcellular fractionation. In addition, we studied the expression of TPP-I in other lysosomal storage disorders such as CLN1, CLN3, muccopolysaccharidoses and GM1 and GM2 gangliosidoses. Our study shows that TPP-I is absent or appears in very small amounts not only in cLINCL subjects with mutations producing severely truncated protein, but also in individuals with missense point mutations, which correlates with loss of TPP-I activity. Of interest, small amounts of TPP-I were detected in lysosomal fraction from fibroblasts from cLINCL subject with protracted form. This observation suggests that the presence of small amounts of TPP-I in lysosomes is able to delay significantly CLN2 disease process. We also show that TPP-I immunoreactivity is increased in the brain tissue of CLN1 and CLN3 subjects, stronger in glial cells and macrophages than neurons. Less prominent increase of TPP-I staining was found in muccopolysaccharidoses and GM1 and GM2 gangliosidoses. These data suggest that TPP-I participates in lysosomal turnover of proteins in pathological conditions associated with cell/tissue injury.

PMID: 11589013 [PubMed - indexed for MEDLINE]

☐ **5:** Ment Retard Dev Disabil Res Rev. 2001;7(3):190-9. Related Articles, Links

Systematic approach to the diagnosis of lysosomal storage disorders.

Weibel TD, Brady RO.

Developmental and Metabolic Neurology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20892-1260, USA.

Disorders that arise as a result of lysosomal dysfunction represent some of the most challenging diagnostic problems in medicine. Not only are these disorders infrequently seen, but they may also present with signs and symptoms that mimic perinatal injury, food intolerance, or the sequellae of neonatal infection. Misidentification can lead to significant delay in diagnosis. Ironically, as the prevailing economic climate places increasing time constraints on practicing physicians, medical research is providing treatment strategies and management techniques that are most effective if applied early in the course of the disease. Most lysosomal storage disorders can now be definitively diagnosed once the signs are recognized. In many cases the benefits of early diagnosis, enlightened management, and appropriate referral are considerable. The aim of this paper is to demystify this elusive class of diseases, to promote clinical vigilance in their detection, and to provide a systematic approach to diagnosis when clinical suspicion is aroused. Copyright 2001 Wiley-Liss, Inc.

Publication Types:
- Review
- Review, Tutorial

PMID: 11553935 [PubMed - indexed for MEDLINE]

☐ **6:** Curr Opin Mol Ther. 2001 Aug;3(4):399-406. Related Articles, Links

Gene therapy for lysosomal storage disorders.

Yew NS, Cheng SH.

Genzyme Corporation, Framingham, MA 01701-9322, USA. nelson.yew@genzyme.com

Deficiencies in one or several of the numerous degradative enzymes that reside in the lysosome often result in one of many clinically severe diseases, almost all of which have no currently available therapy. Although bone marrow transplantation, enzyme replacement and substrate inhibition therapies are being considered, gene therapy represents an increasingly attractive approach, particularly for those lysosomal storage diseases with neurological manifestations. This review summarizes the most recent advances in developing gene therapies for this large and heterogeneous group of disorders.

Publication Types:
- Review
- Review, Tutorial

PMID: 11525564 [PubMed - indexed for MEDLINE]

☐ 7: Southeast Asian J Trop Med Public Health. 1999;30 Suppl 2:111-3. Related Articles, Links

Pilot neonatal screening program for lysosomal storage disorders, using lamp-1.

Ranierri E, Gerace RL, Ravenscroft EM, Hopwood JJ, Meikle PJ.

Department of Chemical Pathology, Women's and Children's Hospital, North Adelaide, Australia.

We have demonstrated that the lysosome associated membrane protein (LAMP-1) is elevated in plasma from approximately 70% of lysosomal storage disorder patients. As part of the development of a newborn screening program for lysosomal storage disorders we have developed a first tier screening assay based upon the level of LAMP-I in blood spots taken from newborn Guthrie cards. To determine the effectiveness of the first-tier marker a prospective pilot Guthrie neonatal screening program for the identification of LSD was commenced in April 1998. Prior to commencement of the pilot program ethical approval was obtained and information leaflets regarding the neonatal screening of LSD were distributed to parents at the time of their infant's Guthrie collection. The LAMP-1 assay utilizes a chicken polyclonal and a mouse monoclonal in a sandwich time resolved fluorescent immunoassay. LAMP-1 blood-spot calibrators and quality control specimens were developed and shown to be stable and reproducible. To date 11,183 infants have been screened using LAMP-1. The population distribution is described with a median and 98th percentile of 220pg/l whole blood and 483microg/l whole blood respectively. Acceptable CV% for intra and inter assay of 8.9% and 10% respectively were obtained.

PMID: 11400745 [PubMed - indexed for MEDLINE]

☐ **8:** Southeast Asian J Trop Med Public Health. 1999;30 Suppl 2:104-10. Related Articles, Links

Newborn screening for lysosomal storage disorders.

Meikle PJ, Ranieri E, Ravenscroft EM, Hua CT, Brooks DA, Hopwood JJ.

Department of Chemical Pathology, Women's and Children's Hospital, North Adelaide, Australia. pmeikle@medicine.adelaide.edu.au

Lysosomal storage disorders (LSD) represent a group of over 40 distinct genetic diseases with a total incidence of approximately 1:7,000 births. Bone marrow transplantation and enzyme replacement therapy are currently in use for the treatment of some disorders and new forms of enzyme and gene replacement therapy are actively being researched. The effectiveness of these therapies, particularly for the LSD involving the central nervous system and bone pathology, will rely heavily upon the early diagnosis and treatment of the disorder, before the onset of irreversible pathology. In the absence of a family history the only practical way to detect these disorders will be by a newborn screening program. One common feature of these disorders is an increase in the number and size of lysosomes within the cell from approximately 1% to as much as 50% of total cellular volume. Associated with this, is a corresponding increase in some lysosomal proteins. We propose that the measurement of one or more of these proteins in blood spots taken from Guthrie cards, will form the basis of a newborn screening program, for the detection of all LSD. We have identified a number of lysosomal proteins as potential markers for LSD. The level of these proteins has been determined in blood spots taken from Guthrie cards and in plasma samples from over 300 LSD affected individuals representing 25 disorders. Based on these results we have proposed a strategy for a newborn screening program involving a two tier system, utilizing time resolved fluorescence immunoquantification of the protein markers in the first tier, followed by tandem mass spectrometry for the determination of stored substrates in the second tier assays.

PMID: 11400743 [PubMed - indexed for MEDLINE]

☐ **9:** Clin Chem. 2000 Sep;46(9):1318-25. Related Articles, Links

Determination of acid alpha-glucosidase protein: evaluation as a screening marker for Pompe disease and other lysosomal storage disorders.

Umapathysivam K, Whittle AM, Ranieri E, Bindloss C, Ravenscroft EM, van Diggelen OP, Hopwood JJ, Meikle PJ.

Lysosomal Diseases Research Unit and State Screening Services, Department of Chemical Pathology, Women's and Children's Hospital, 72 King William Rd., North Adelaide, South Australia 5006, Australia.

BACKGROUND: In recent years, there have been significant advances in the development of enzyme replacement and other therapies for lysosomal storage disorders (LSDs). Early diagnosis, before the onset of irreversible pathology, has been demonstrated to be critical for maximum efficacy of current and proposed therapies. In the absence of a family history, the presymptomatic detection of these disorders ideally can be achieved through a newborn screening program. One approach to the development of such a program is the identification

of suitable screening markers. In this study, the acid alpha-glucosidase protein was evaluated as a marker protein for Pompe disease and potentially for other LSDs. METHODS: Two sensitive immunoquantification assays for the measurement of total (precursor and mature) and mature forms of acid alpha-glucosidase protein were used to determine the concentrations in plasma and dried blood spots from control and LSD-affected individuals. RESULTS: In the majority of LSDs, no significant increases above control values were observed. However, individuals with Pompe disease showed a marked decrease in acid alpha-glucosidase protein in both plasma and whole blood compared with unaffected controls. For plasma samples, this assay gave a sensitivity of 95% with a specificity of 100%. For blood spot samples, the sensitivity was 82% with a specificity of 100%. CONCLUSIONS: This study demonstrates that it is possible to screen for Pompe disease by screening the concentration of total acid alpha-glucosidase in plasma or dried blood spots.

PMID: 10973860 [PubMed - indexed for MEDLINE]

☐ **10:** J Am Soc Nephrol. 2000 Aug;11(8):1542-7. Related Articles, Links

Full text article at www.jasn.org

Gene therapy for lysosomal storage disorders with neuropathology.

Ioannou YA.

Department of Human Genetics, Gene Therapy and Molecular Medicine, The Mount Sinai School of Medicine, New York, NY 10029-6574, USA. ioanny01@doc.mssm.edu

Publication Types:
- Review
- Review, Tutorial

PMID: 10906169 [PubMed - indexed for MEDLINE]

☐ **11:** Clin Chem. 2000 Feb;46(2):167-74. Related Articles, Links

FREE full text article at www.clinchem.org

Saposins A, B, C, and D in plasma of patients with lysosomal storage disorders.

Chang MH, Bindloss CA, Grabowski GA, Qi X, Winchester B, Hopwood JJ, Meikle PJ.

Lysosomal Diseases Research Unit, Department of Chemical Pathology, Women's and Children's Hospital, 72 King William Road, North Adelaide, South Australia 5006, Australia.

BACKGROUND: Early diagnosis of lysosomal storage disorders (LSDs), before the onset of irreversible pathology, will be critical for maximum efficacy of many current and proposed therapies. To search for potential markers of LSDs, we measured saposins A, B, C, and D in patients with these disorders. METHODS: Four time-delayed fluorescence

immunoquantification assays were used to measure each of the saposins in plasma from 111 unaffected individuals and 334 LSD-affected individuals, representing 28 different disorders. RESULTS: Saposin A was increased above the 95th centile of the control population in 59% of LSD patients; saposins B, C, and D were increased in 25%, 61%, and 57%, respectively. Saposins were increased in patients from several LSD groups that in previous studies did not show an increase of lysosome-associated membrane protein-1 (LAMP-1). CONCLUSION: Saposins may be useful markers for LSDs when used in conjunction with LAMP-1.

PMID: 10657372 [PubMed - indexed for MEDLINE]

☐ **12:** Adv Pediatr. 1999;46:409-40. Related Articles, Links

Hydrops fetalis: lysosomal storage disorders in extremis.

Stone DL, Sidransky E.

Clinical Neuroscience Branch, National Institute of Mental Health, Bethesda, Maryland, USA.

In recent years there has been an increased recognition that hydrops fetalis may be an extreme presentation of many of the lysosomal storage disorders. Hydrops fetalis, the excessive accumulation of serous fluid in the subcutaneous tissues and serous cavities of the fetus, has many possible etiologies, providing a diagnostic challenge for the physician. Ten different lysosomal storage disorders have now been diagnosed in infants with hydrops fetalis, including mucopolysaccharidosis (MPS) VII and IVA, type 2 Gaucher disease, sialidosis, GMI gangliosidosis, galactosialidosis, Niemann-Pick disease type C, disseminated lipogranulomatosis (Farber disease), infantile free sialic acid storage disease (ISSD), and mucolipidosis II (I-cell disease). Frequently, these inborn errors of metabolism are recognized only after the unfortunate recurrence of hydrops fetalis in several pregnancies of a family. Making the diagnosis relies on the physician having a high index of suspicion and ordering appropriate testing, which can often be performed prenatally. In several of these disorders, including MPS VII, infantile galactosialidosis, type 2 Gaucher disease, and ISSD, hydrops fetalis is a relatively common presentation. A greater physician awareness of hydrops fetalis as a presentation of lysosomal disease will facilitate establishing a diagnosis in cases that would have previously been considered idiopathic and will enable a better estimation of the incidence of this association. Lysosomal disorders are among the few causes of nonimmune hydrops fetalis in which an accurate recurrence risk can be ascertained. With an early and accurate diagnosis, genetic counseling and family planning can be offered in these difficult cases.

Publication Types:
- Review
- Review, Tutorial

PMID: 10645471 [PubMed - indexed for MEDLINE]

☐ **13:** Pediatr Res. 1999 Nov;46(5):501-9. Related Articles, Links

Alpha-mannosidosis in the guinea pig: a new animal model for lysosomal storage disorders.

Crawley AC, Jones MZ, Bonning LE, Finnie JW, Hopwood JJ.

Department of Chemical Pathology, Women's and Children's Hospital, North Adelaide, SA, Australia.

Alpha-mannosidosis is a lysosomal storage disorder resulting from deficient activity of lysosomal alpha-mannosidase. It has been described previously in humans, cattle, and cats, and is characterized in all of these species principally by neuronal storage leading to progressive mental deterioration. Two guinea pigs with stunted growth, progressive mental dullness, behavioral abnormalities, and abnormal posture and gait, showed a deficiency of acidic alpha-mannosidase activity in leukocytes, plasma, fibroblasts, and whole liver extracts. Fractionation of liver demonstrated a deficiency of lysosomal (acidic) alpha-mannosidase activity. Thin layer chromatography of urine and tissue extracts confirmed the diagnosis by demonstrating a pattern of excreted and stored oligosaccharides almost identical to that of urine from a human alpha-mannosidosis patient. Widespread neuronal vacuolation was observed throughout the CNS, including the cerebral cortex, hippocampus, thalamus, cerebellum, midbrain, pons, medulla, and the dorsal and ventral horns of the spinal cord. Lysosomal vacuolation also occurred in many other visceral tissues and was particularly severe in pancreas, thyroid, epididymis, and peripheral ganglion. Axonal spheroids were observed in some brain regions, but gliosis and demyelination were not observed. Ultrastructurally, most vacuoles in both the CNS and visceral tissues were lucent or contained fine fibrillar or flocculent material. Rare large neurons in the cerebral cortex contained fine membranous structures. Skeletal abnormalities were very mild. Alpha-mannosidosis in the guinea pig closely resembles the human disease and will provide a convenient model for investigation of new therapeutic strategies for neuronal storage diseases, such as enzyme replacement and gene replacement therapies.

PMID: 10541310 [PubMed - indexed for MEDLINE]

☐ **14:** Biochim Biophys Acta. 1999 Oct 8;1455(2-3):69-84. Related Articles, Links

Glycoprotein lysosomal storage disorders: alpha- and beta-mannosidosis, fucosidosis and alpha-N-acetylgalactosaminidase deficiency.

Michalski JC, Klein A.

Laboratoire de Chimie Biologique, UMR 8576 CNRS (UMR 111 CNRS), Universite des Sciences et Technologies de Lille, Villeneuve d'Ascq, France. jean-claude.michalski@univ-lille1.fr

Glycoproteinoses belong to the lysosomal storage disorders group. The common feature of these diseases is the deficiency of a lysosomal protein that is part of glycan catabolism. Most of the lysosomal enzymes involved in the hydrolysis of glycoprotein carbohydrate chains are exo-glycosidases, which stepwise remove terminal monosaccharides. Thus, the deficiency of a single enzyme causes the blockage of the entire pathway and induces a storage of incompletely degraded substances inside the lysosome. Different mutations may be observed in a single disease and in all cases account for the nonexpression of lysosomal glycosidase activity. Different clinical phenotypes generally characterize a specific disorder,

which rather must be described as a continuum in severity, suggesting that other biochemical or environmental factors influence the course of the disease. This review provides details on clinical features, genotype-phenotype correlations, enzymology and biochemical storage of four human glycoprotein lysosomal storage disorders, respectively alpha- and beta-mannosidosis, fucosidosis and alpha-N-acetylgalactosaminidase deficiency. Moreover, several animal disorders of glycoprotein metabolism have been found and constitute valuable models for the understanding of their human counterparts.

Publication Types:
- Review
- Review, Tutorial

PMID: 10571005 [PubMed - indexed for MEDLINE]

☐ **15:** Exp Mol Pathol. 1999 Jun;66(2):123-30. Related Articles, Links

Comment in:
- Exp Mol Pathol. 2001 Apr;70(2):173-4.

ELSEVIER SCIENCE
FULL-TEXT ARTICLE

Molecular characterization of a novel endonuclease (Xib) and possible involvement in lysosomal glycogen storage disorders.

Malferrari G, Mazza U, Tresoldi C, Rovida E, Nissim M, Mirabella M, Servidei S, Biunno I.

Istituto Tecnologie Biomediche Avanzate-Consiglio Nazionale delle Ricerche, Via Fratelli Cervi 93, Segrate Milano, 20090, Italy.

We cloned and partially characterized a human endonuclease (Xib) which shows sequence homologies to pancreatic DNase I but an enzymatic activity closer to DNase II. We report on the structural differences found between Xib and other recently cloned human DNases. Fluores cence microscopy analysis of transiently transfected cells with Xib::pEGFP constructs indicate that the protein is located in the cytoplasm and possibly anchored to a membrane, as deduced from a hydrophobic amino acid stretch present at the C-terminal end. Xib is overexpressed in muscle and cardiac tissues and is alternately spliced in several normal and neoplastic cells. In situ hybridization studies using human cardiac and muscle biopsies indicate accumulation of Xib transcript in the vacuoles of muscle cells from patients affected by vacuolar myopathy as acid maltase deficiency; however, no point mutations were detected in their DNA.

PMID: 10409440 [PubMed - indexed for MEDLINE]

☐ **16:** Neurochem Res. 1999 Apr;24(4):601-15. Related Articles, Links

Gene transfer approaches to the lysosomal storage disorders.

Barranger JA, Rice EO, Swaney WP.

Human Genetics Department at the University of Pittsburgh, PA 15261, USA.

The work summarized in this paper used animal and cell culture models systems to develop gene therapy approaches for the lysosomal storage disorders. The results have provided the scientific basis for a clinical trial of gene transfer to hematopoietic stem cells (HSC) in Gaucher disease which is now in progress. The clinical experiment is providing evidence of HSC transduction, competitive engraftment of genetically corrected HSC, expression of the GC transgene, and the suggestion of a clinical response. In this paper we will review the progress made in Gaucher disease and include how gene transfer might be studied in other lysosomal storage disorders.

Publication Types:
- Review
- Review, Tutorial

PMID: 10227692 [PubMed - indexed for MEDLINE]

☐ **17:** JAMA. 1999 Jan 20;281(3):249-54. Related Articles, Links

JAMA

Prevalence of lysosomal storage disorders.

Meikle PJ, Hopwood JJ, Clague AE, Carey WF.

Department of Chemical Pathology, Women's and Children's Hospital, Adelaide, Australia. p.meikle@medicine.adelaide.edu.au

CONTEXT: Lysosomal storage disorders represent a group of at least 41 genetically distinct, biochemically related, inherited diseases. Individually, these disorders are considered rare, although high prevalence values have been reported in some populations. These disorders are devastating for individuals and their families and result in considerable use of resources from health care systems; however, the magnitude of the problem is not well defined. To date, no comprehensive study has been performed on the prevalence of these disorders as a group. OBJECTIVE: To determine the prevalence of lysosomal storage disorders individually and as a group in the Australian population. DESIGN: Retrospective case studies. SETTING: Australia, from January 1, 1980, through December 31, 1996. MAIN OUTCOME MEASURE: Enzymatic diagnosis of a lysosomal storage disorder. RESULTS: Twenty-seven different lysosomal storage disorders were diagnosed in 545 individuals. The prevalence ranged from 1 per 57000 live births for Gaucher disease to 1 per 4.2 million live births for sialidosis. Eighteen of 27 disorders had more than 10 diagnosed cases. As a group of disorders, the combined prevalence was 1 per 7700 live births. There was no significant increase in the rate of either clinical diagnoses or prenatal diagnoses of lysosomal storage disorders during the study period. CONCLUSIONS: Individually, lysosomal storage disorders are rare genetic diseases. However, as a group, they are relatively common and represent an important health problem in Australia.

PMID: 9918480 [PubMed - indexed for MEDLINE]

☐ **18:** FEBS Lett. 1998 Dec 28;441(3):369-72. Related Articles, Links

ELSEVIER SCIENCE
FULL-TEXT ARTICLE

Application of magnetic chromatography to the isolation of lysosomes from fibroblasts of patients with lysosomal storage disorders.

Diettrich O, Mills K, Johnson AW, Hasilik A, Winchester BG.

Institute of Child Health, London, UK. o.diettrich@ich.ucl.ac.uk

A method for the purification of lysosomes from fibroblasts has been developed which uses endocytosis of superparamagnetic colloidal iron dextran particles followed by separation of the iron-containing lysosomes in a magnetic field. This permitted isolation of lysosomes from fibroblasts from patients with infantile sialic acid storage disorder and other lysosomal storage diseases in which a shift in lysosomal density induced by the storage material prevents purification by centrifugation in a Percoll gradient. The magnetic lysosomes isolated from these cells are very similar to those from normal cells as judged by lysosomal marker enzyme activity and 2D-PAGE analysis of the enriched proteins.

PMID: 9891973 [PubMed - indexed for MEDLINE]

☐ **19:** Clin Chem. 1998 Oct;44(10):2094-102. Related Articles, Links

FREE full text article at
www.clinchem.org

Evaluation of the lysosome-associated membrane protein LAMP-2 as a marker for lysosomal storage disorders.

Hua CT, Hopwood JJ, Carlsson SR, Harris RJ, Meikle PJ.

Department of Chemical Pathology, Women's and Children's Hospital, North Adelaide, South Australia, Australia.

For many lysosomal storage disorders, presymptomatic detection, before the onset of irreversible pathology, will greatly improve the efficacy of current and proposed therapies. In the absence of a family history, presymptomatic detection can be achieved only by a comprehensive newborn screening program. Recently we reported that the lysosome-associated membrane protein LAMP-1 was increased in the plasma from approximately 70% of individuals with lysosomal storage disorders. Here we report on the evaluation of a second lysosome-associated membrane protein, LAMP-2, as a marker for this group of disorders. The median concentration of LAMP-2 in the plasma of healthy individuals was 1.21 mg/L, fourfold higher than the median LAMP-1 concentration (0.31 mg/L). LAMP-2 was increased in >66% of patients with lysosomal storage disorders, and the increases coincided with increased LAMP-1 concentrations. The reference intervals for LAMP-1 and LAMP-2 in blood spots taken from newborns were 0.20-0.54 mg/L (n = 1600) and 0.95-3.06 mg/L (n = 1600), respectively. A high correlation was observed between the concentrations of LAMP-1 and LAMP-2 in both control and affected individuals. The higher concentrations of LAMP-2, relative to LAMP-1, in plasma make LAMP-2 an attractive marker; however, the final selection will be dependent on the availability of new diagnostic markers and their ability to detect disorders currently not identified by LAMP-2.

PMID: 9761240 [PubMed - indexed for MEDLINE]

☐ **20:** Ryoikibetsu Shokogun Shirizu. 1998;(19 Pt 2):601-5. Related Articles, Links

[Lysosomal transport disorders: cystinosis and sialic acid storage disorders]

[Article in Japanese]

Eto Y.

Department of Pediatrics, Tokyo Jikei University School of Medicine.

Publication Types:
- Review
- Review, Tutorial

PMID: 9645145 [PubMed - indexed for MEDLINE]

☐ **21:** Brain Pathol. 1998 Jan;8(1):175-93. Related Articles, Links

Cellular pathology of lysosomal storage disorders.

Walkley SU.

Department of Neuroscience, Rose F. Kennedy Center for Research in Mental Retardation and Human Development, Albert Einstein College of Medicine, Bronx, NY 10461, USA. walkley@aecom.yu.edu

Lysosomal storage disorders are rare, inborn errors of metabolism characterized by intralysosomal accumulation of unmetabolized compounds. The brain is commonly a central focus of the disease process and children and animals affected by these disorders often exhibit progressively severe neurological abnormalities. Although most storage diseases result from loss of activity of a single enzyme responsible for a single catabolic step in a single organelle, the lysosome, the overall features of the resulting disease belies this simple beginning. These are enormously complex disorders with metabolic and functional consequences that go far beyond the lysosome and impact both soma-dendritic and axonal domains of neurons in highly neuron type-specific ways. Cellular pathological changes include growth of ectopic dendrites and new synaptic connections and formation of enlargements in axons far distant from the lysosomal defect. Other storage diseases exhibit neuron death, also occurring in a cell-selective manner. The functional links between known molecular genetic and enzyme defects and changes in neuronal integrity remain largely unknown. Future studies on the biology of lysosomal storage diseases affecting the brain can be anticipated to provide insights not only into these pathogenic mechanisms, but also into the role of lysosomes and related organelles in normal neuron function.

Publication Types:
- Review
- Review, Tutorial

PMID: 9458175 [PubMed - indexed for MEDLINE]

☐ **22:** Clin Chem. 1997 Aug;43(8 Pt 1):1325-35. Related Articles, Links

FREE full text article at www.clinchem.org

Diagnosis of lysosomal storage disorders: evaluation of lysosome-associated membrane protein LAMP-1 as a diagnostic marker.

Meikle PJ, Brooks DA, Ravenscroft EM, Yan M, Williams RE, Jaunzems AE, Chataway TK, Karageorgos LE, Davey RC, Boulter CD, Carlsson SR, Hopwood JJ.

Department of Chemical Pathology, Women's and Children's Hospital, North Adelaide, Australia. omeikle@medicine.adelaide.edu.au

Early diagnosis of lysosomal storage disorders (LSDs), before the onset of irreversible pathologies, will be a key factor in the development of effective therapies for many of these disorders. Newborn screening offers a potential mechanism for the early detection of these disorders. From studies of both normal and LSD-affected human skin fibroblasts we identified the lysosome-associated membrane protein LAMP-1 as a potential diagnostic marker. We have developed a sensitive method for the quantification of this protein with a time-resolved fluorescence immunoassay. A soluble form of LAMP-1 was observed in plasma samples, and determination of 152 unaffected individuals gave a median value of 303 micrograms/L with the 5th and 95th percentile at 175 and 448 micrograms/L respectively. Plasma samples from 320 LSD-affected individuals representing 25 different disorders were assayed. We observed that 17 of the 25 disorder groups tested had > 88% of individuals above the 95th percentile of the control population, with 12 groups having 100% above the 95th percentile. Overall, 72% of patients had LAMP-1 concentrations above the 95th percentile of the unpartitioned control population. We suggest that LAMP-1 may be a useful marker in newborn screening for LSDs.

PMID: 9267309 [PubMed - indexed for MEDLINE]

☐ **23:** Exp Cell Res. 1997 Jul 10;234(1):85-97. Related Articles, Links

ELSEVIER SCIENCE FULL-TEXT ARTICLE

Lysosomal biogenesis in lysosomal storage disorders.

Karageorgos LE, Isaac EL, Brooks DA, Ravenscroft EM, Davey R, Hopwood JJ, Meikle PJ.

Department of Chemical Pathology, Women's and Children's Hospital, North Adelaide, South Australia.

Lysosomal biogenesis is an orchestration of the structural and functional elements of the lysosome to form an integrated organelle and involves the synthesis, targeting, functional residence, and turnover of the proteins that comprise the lysosome. We have investigated lysosomal biogenesis during the formation and dissipation of storage vacuoles in two model systems. One involves the formation of sucrosomes in normal skin fibroblasts and the other utilizes storage disorder-affected skin fibroblasts; both of these systems result in an increase in the size and the number of lysosomal vacuoles. Lysosomal proteins, beta-hexosaminidase, alpha-mannosidase, N-acetylgalactosamine-4-sulfatase, acid phosphatase,

and the lysosome-associated membrane protein, LAMP-1, were shown to be elevated between 2- and 28-fold above normal during lysosomal storage. Levels of mRNA for the lysosome-associated membrane proteins LAMP-1 and LAMP-2, N-acetylgalactosamine-4-sulfatase, and the 46- and 300-kDa mannose-6-phosphate receptors were also elevated 2- to 8-fold. The up-regulation of protein and mRNA lagged 2-4 days behind the formation of lysosomal storage vacuoles. Correction of storage, in both systems, resulted in the rapid decline of the mRNA to basal levels, with a slower decrease in the levels of lysosomal proteins. Lysosomal biogenesis in storage disorders is shown to be a regulated process which is partially controlled at, or prior to, the level of mRNA. Although lysosomal proteins were differentially regulated, the coordination of these events in lysosomal biogenesis would suggest that a common mechanism(s) may be in operation.

PMID: 9223373 [PubMed - indexed for MEDLINE]

☐ **24:** Nippon Rinsho. 1995 Dec;53(12):3068-71. Related Articles, Links

[Lysosomal membrane transport disorders--cystinosis and sialic acid storage disorders (Salla disease, ISSD)]

[Article in Japanese]

Yano T, Ohno K.

Department of Neurobiology School of Life Science, Tottori University, Faculty of Medicine.

Cystinosis and sialic acid storage diseases (Salla disease, ISSD; infantile sialic acid storage disease) are lysosomal membrane disorders resulting from defective carrier-mediated transport of cystine and sialic acid across the lysosomal membrane. Both are rare autosomal recessively inherited disorders. The major clinical manifestations of cystinosis are renal failure and ocular damages. Sialic acid storage diseases are characterized by various degrees of psychomotor retardation. Salla disease patients trace a mild clinical course, and the life span is relatively long. While, in patients with ISSD follow a very severe progressive clinical course and often die in the first year of life. The genes responsible for each disease have not been isolated, the etiologies are not well known, and there is no specific treatment.

Publication Types:
- Review
- Review, Tutorial

PMID: 8577060 [PubMed - indexed for MEDLINE]

☐ **25:** Pediatr Pol. 1995 Oct;70(10):847-55. Related Articles, Links

[Thin-layer chromatography of urine oligosaccharides in diagnosis of some lysosomal storage disorders]

[Article in Polish]

Lugowska A, Tylki-Szymanska A, Sawnor-Korszynska D.

Zaklad Diagnostyki Laboratoryjnej Centrum Zdrowia Dziecka w Warszawie.

Inherited lysosomal storage disorders are caused by the deficiency or importantly lowered activity of one of the lysosomal enzymes, leading to the storage in the lysosomes the not degraded high-molecular substrates, among others: mucopolysaccharides, glycolipids, oligosaccharides and glycoproteins. Thin-layer chromatography of urine oligosaccharides allows reliable and fast diagnosis of some lysosomal storage disorders e.g. alpha-mannosidosis, fucosidosis, sialidosis, galactosialidosis, Schindler disease, GM1-gangliosidosis, GM2-gangliosidosis (Sandhoff type), Pompe disease, Salla disease, mucolipidosis II and III. We are presenting a modification of the Humbel and Collart's method of TLC of urine oligosaccharides. The principle of our modification is to introduce of the preliminary desalting step of the urine on the columns containing anionit BioRad AG 1 x 8 and cationit Dowex 50 x 8-200.

PMID: 8649932 [PubMed - indexed for MEDLINE]

26: Am J Hum Genet. 1995 Oct;57(4):893-901. Related Articles, Links

Lysosomal free sialic acid storage disorders with different phenotypic presentations--infantile-form sialic acid storage disease and Salla disease--represent allelic disorders on 6q14-15.

Schleutker J, Leppanen P, Mansson JE, Erikson A, Weissenbach J, Peltonen L, Aula P.

Department of Medical Genetics, University of Turku, Finland.

Similarities in biochemical findings have suggested that Salla disease (SD) and the infantile form of sialic acid storage disease (ISSD) could represent allelic disorders, despite their drastically different clinical phenotypes. SD and ISSD are both characterized by lysosomal storage of free N-acetyl neuraminic acid. However, in SD the increase detected in urine is 8-24-fold, whereas in ISSD the corresponding amount is 20-50-fold and patients are also more severely affected. Here we report linkage studies in 50 Finnish SD families and 26 non-Finnish families with no genealogical connections to Finns affected either with the Finnish type of SD, the "intermediate" form of the disease, or ISSD. All forms of the disease show linkage to the same locus on 6q14-q15. Haplotype analyses of Finnish SD chromosomes revealed one common haplotype, which was also seen in most of the non-Finnish patients with Finnish type of SD. This ancestral haplotype deviated from those observed in ISSD patients, who had a different common haplotype.

PMID: 7573051 [PubMed - indexed for MEDLINE]

27: J Inherit Metab Dis. 1995;18(6):717-22. Related Articles, Links

Elevated plasma chitotriosidase activity in various lysosomal storage disorders.

Guo Y, He W, Boer AM, Wevers RA, de Bruijn AM, Groener JE, Hollak CE, Aerts JM, Galjaard H, van Diggelen OP.

Department of Clinical Genetics, Erasmus University, Rotterdam, The Netherlands.

Recently a striking elevation of the activity of chitotriosidase, an endo beta-glucosaminidase distinct from lysozyme, was found in plasma from patients with Gaucher type I disease (McKusick 230800). Plasma chitotriosidase originates from activated macrophages and this elevation is secondary to the basic defect in Gaucher disease. To investigate the specificity of this phenomenon, we have investigated 24 different lysosomal storage diseases. In 11 different diseases increased chitotriosidase activity in plasma was found (in 28% of the patients). None of these diseases showed elevations as high as in Gaucher disease. Chitotriosidase was not significantly elevated in plasma from 20 different non-lysosomal enzymopathies or in plasma from patients with infectious diseases associated with hepatomegaly. The results show that marked elevation of chitotriosidase activity in plasma appears to be specific for Gaucher disease. The data further suggest that elevated levels of chitotriosidase activity in plasma from patients with unexplained diseases may be indicative for a lysosomal disorder.

PMID: 8750610 [PubMed - indexed for MEDLINE]

☐ **28:** Southeast Asian J Trop Med Public Health. 1995;26 Suppl 1:54-8. Related Articles, Links

Lysosomal storage disorders in Thailand: the Siriraj experience.

Wasant P, Wattanaweeradej S, Raksadawan N, Kolodny EH.

Department of Pediatrics, Siriraj Hospital Medical School, Mahidol University, Bangkok, Thailand.

Lysosomal storage disorders are a heterogeneous group of biochemical genetic disorders; currently 40-50 are known. The clinical phenotype is determined by the tissue distribution of the storage material and degree of enzyme deficiency. The genetic transmission is mostly autosomal recessive. Lysosomal storage disorders can be divided into three groups according to the major organ system pathology: (1) Primary involvement of the central nervous system without significant somatic or skeletal pathology. Disorders of grey matter, eg gangliosidosis and disorders of white matter eg the leucodystrophy are the most common; (2) Primary involvement of the reticuloendothelial system with or without associated neuropathology, eg Niemann-Pick disease and Gaucher disease; (3) Multisystem involvement in which skeletal manifestations are prominent features. The mucopolysaccharidosis and mucolipidoses are the two major forms with this clinical phenotype. Lysosomal storage disorders identified at Siriraj Hospital are neuronal ceroid lipofuscinosis, GMI gangliosidosis, mucolipidosis II, Maroteaux-Lamy, sialidosis, Sly syndrome, Hunter syndrome, Morquio syndrome, Gaucher disease, Niemann-Pick, Sandhoff disease, Pompe's disease and many more. Most patients came from the provinces where consanguinity is common. Confirmation usually is done by enzyme assays using skin fibroblast culture or leucocytes. Genetic counseling is extremely important and prenatal diagnosis is recommended to high-risk couple.

Publication Types:
- Case Reports

PMID: 8629143 [PubMed - indexed for MEDLINE]

☐ **29:** Br Med Bull. 1995 Jan;51(1):106-22. Related Articles, Links

Gene therapy of lysosomal storage disorders.

Salvetti A, Heard JM, Danos O.

Retrovirus et Transfert Genetique, Institut Pasteur, Paris, France.

Lysosomal storage disorders (LSD) result from deficiencies in enzymes normally implicated in the catabolism of macromolecules inside the lysosome. Many of these enzymes can reach the lysosome after being secreted in the extracellular medium and recaptured by specific cell surface receptors. This has suggested a rationale for therapeutic approaches in LSD, in which the missing enzyme is provided by an external source. Current therapies based on this concept, including the administration of purified enzyme and bone marrow transplantation, have been shown to result in clinical improvements in both animal models and patients. Although considerable difficulties must be surmounted, LSD present a favourable situation for gene therapy. The gene corresponding to the affected enzyme has been identified in most diseases and cDNAs are available. Low and unregulated levels of enzyme activity should be sufficient for correction. Importantly, a variety of gene transfer strategies can be carefully evaluated in animal models.

Publication Types:
- Review
- Review, Academic

PMID: 7767637 [PubMed - indexed for MEDLINE]

☐ **30:** Biochim Biophys Acta. 1994 May 25;1226(2):138-44. Related Articles, Links

Free sphingoid bases in tissues from patients with type C Niemann-Pick disease and other lysosomal storage disorders.

Rodriguez-Lafrasse C, Rousson R, Pentchev PG, Louisot P, Vanier MT.

Department of Biochemistry, INSERM-CNRS 189, Lyon-Sud Medical School, Oullins, France.

The 20-fold increase of free sphingoid bases found in liver from a murine model of Niemann-Pick type C (NPC) combined to the NPC-like phenotype induced by addition of sphinganine to normal fibroblast cultures prompted us to investigate the potential involvement of these compounds in the human disease. The contents of sphingosine and sphinganine were measured in liver, spleen, brain and skin fibroblast cultures by a sensitive HPLC method. In liver and spleen from NPC patients, a 6- to 24-fold elevation of sphingosine and sphinganine already prominent at the fetal stage of the disease was observed, while no clear increase could be evidenced in brain tissue. A significant increase, not modulated by the intralysosomal content of free cholesterol, also occurred in skin

fibroblast cultures. To investigate the specificity of these findings, other lysosomal storage disorders were studied. A striking accumulation was found in liver and spleen (24- to 36-fold) from patients with Niemann-Pick disease type A and B (sphingomyelinase-deficient forms), and in cerebral cortex of type A Niemann-Pick disease. A significant storage also occurred in Sandhoff disease, while several other sphingolipidoses showed a moderate elevation. In all cases but Sandhoff disease brain, the sphingosine/sphinganine ratio remained unchanged, suggesting that the accumulated free sphingoid bases derived from sphingolipid catabolism. Formation of complexes between sphingosine and the lipid material accumulated in lysosomes might be a general mechanism in lysosomal lipidoses. In NPC, however, an increase of free sphingoid bases disproportionate to the degree of lysosomal storage and a specific involvement of cultured fibroblasts suggested a more complex or combined mechanism.

PMID: 8204660 [PubMed - indexed for MEDLINE]

☐ **31:** Biol Cell. 1994;81(2):143-52. Related Articles, Links

Effects of suramin, a polyanionic drug inducing lysosomal storage disorders on tooth germs in vitro.

Gritli-Linde A, Ruch JV, Mark MP, Lecolle S, Goldberg M.

Laboratoire de Biologie et Biomateriaux du Milieu Buccal et Osseux, Faculte de Chirurgie Dentaire LA 1505, Montrouge, France.

Suramin, a potent inhibitor of lysosomal enzymes, is commonly employed as a tool for inducing experimental mucopolysaccharidosis and lipidosis. The effects of the drug on embryonic mouse molars were analysed. Presecretory ameloblasts and odontoblasts were loaded with lysosome-like vacuoles. Staining with MC22-33F, an antibody to choline phospholipids and sphingomyelin, was completely reversed in the suramin-treated germs, in that it stained only presecretory ameloblasts (versus odontoblasts and some pulpal cells in the control group), according to a developmentally regulated pattern. The suramin-induced cytoplasmic changes were reminiscent of the features of mucopolysaccharidoses and lipidoses. The basement membrane, separating the enamel organ from the dental papilla, displayed suramin-induced patches, and in predentin collagen fibrillogenesis was found to be disturbed. Furthermore, autoradiography was employed to reveal uptake and distribution of [3H] suramin in the cells and predentin. Finally, a suramin-induced disturbance of the metabolism of sulphated macromolecules was found. The results imply that suramin effects in vitro on tooth germs can be used as a useful experimental model with to study both the action of the drug as well as cell and extracellular matrix perturbations in a mucopolysaccharidosis-like condition.

PMID: 7849606 [PubMed - indexed for MEDLINE]

☐ **32:** Nippon Rinsho. 1993 Sep;51(9):2264-8. Related Articles, Links

[Lysosomal storage disease: a group of genetic neurodegenerative disorders]

[Article in Japanese]

Suzuki Y.

Tokyo Metropolitan Institute of Medical Science.

Lysosomal storage disease is a group of neurometabolic diseases mainly occurring in infancy and childhood. They were first recognized as new diseases on the basis of unique clinical manifestations or pathological findings, and then the stage of biochemical analysis of storage material and enzyme assays in tissues and cells from patients followed. Recent technological development has enabled us to look further into the molecular genetic basis of these inherited diseases. Protein analysis revealed intracellular events of the mutant enzyme molecule responsible for the pathogenesis of a disease, and more detailed information has been obtained about the mutant gene and its product. Clinical manifestations are not always uniform for a single disease with mutations in the same gene. Clinical subtypes have been proposed for many lysosomal diseases. At present, the molecular and metabolic basis of each phenotypic expression is not clear, although common mutations have been found for specific clinical forms in some diseases. In this article, the current status of lysosomal disease research was summarized, particularly focusing on molecular pathology and molecular diagnosis. Finally future prospects for pathogenetic analysis of neural dysfunction and possible gene therapy were briefly discussed.

Publication Types:
- Review
- Review Literature

PMID: 8411700 [PubMed - indexed for MEDLINE]

☐ **33:** J Inherit Metab Dis. 1993;16(2):288-91. Related Articles, Links

Pathogenesis of lysosomal storage disorders as illustrated by Gaucher disease.

Aerts JM, Van Weely S, Boot R, Hollak CE, Tager JM.

E.C. Slater Institute for Biochemical Research, University of Amsterdam, The Netherlands.

Publication Types:
- Review
- Review, Tutorial

PMID: 8411983 [PubMed - indexed for MEDLINE]

☐ **34:** C R Seances Soc Biol Fil. 1993;187(5):596-607. Related Articles, Links

[Lysosomal storage diseases, genetic or drug-induced? effect of glycosaminoglycan and sphingolipid disorders on dental tissues]

[Article in French]

Goldberg M, Gritli A, Bloch-Zupan A, Septier D, Lecolle S, Legrand JM, Ruch JV.

Laboratoire de Biologie et Biomateriaux du Milieu Buccal et Osseux, Faculte de Chirurgie Dentaire, Paris V, Montrouge, France.

In vivo studies were carried out on dental tissues of rat incisor after a single injection of suramin, a drug which induces mucopolysaccharidosis-like disease. Accumulation of lysosome-like structures was seen in secretory ameloblasts and odontoblasts. In vitro studies on embryonic tooth germ buds showed similar changes when they were cultured in presence of suramin. Anti-phospholipid immunolabelling revealed a developmentally regulated temporo-spatial pattern. Radiolabeling with 3H-suramin indicated cytosolic and nuclear incorporation. The drug acting as polyanion interacted directly with predentine. 35S sulphate incorporation was impaired by the drug. Another lysosomal storage disease, the sphingolipidosis, Krabbe's disease was also investigated in human. Changes were observed in pulp cells and as a consequence in dentin. Enamel also displayed many changes. Pharmacological or genetically acquired diseases constitute models providing insights on the role played by glycosaminoglycans and phospholipids in biomineralization.

PMID: 8069712 [PubMed - indexed for MEDLINE]

☐ **35:** Clin Neuropathol. 1992 Sep-Oct;11(5):251-5. Related Articles, Links

Neuronal ubiquitin and neurofilament expression in different lysosomal storage disorders.

Zhan SS, Beyreuther K, Schmitt HP.

Institute of Neuropathology, University of Heidelberg, Germany.

We studied various lysosomal storage disorders such as Tay-Sachs' disease, Niemann-Pick's disease, and Hunter's disease for their immunoreactivity with antibodies against ubiquitin (Ub) and neurofilaments (NF). We found that in all cases, irrespective of the nature of the storage material or disorder, only a minor proportion of neurons (20-30% at most), as a rule, moderately reacted with the Ub antibody, while the majority of the distended neurons neither expressed Ub nor NF epitopes. These findings suggest that the UB dependent proteolytic pathway may play a secondary role in the lysosomal storage disorders, at least in the advanced stages which are observed at autopsy. It seems that the Ub expression of a minor proportion of neurons should be regarded as an unspecific epiphenomenon rather than as a mechanism of major significance in the basic metabolism of these disorders, in which the inclusions consist of membrane-bound lipid material.

PMID: 1385029 [PubMed - indexed for MEDLINE]

☐ **36:** FASEB J. 1991 Mar 1;5(3):301-8. Related Articles, Links

Saposin proteins: structure, function, and role in human lysosomal storage disorders.

O'Brien JS, Kishimoto Y.

Department of Neurosciences, University of California, San Diego, La Jolla 92093.

Saposins are sphingolipid activator proteins, four of which are derived from a single precursor, prosaposin, by proteolytic processing. These small heat-stable glycoproteins (12-14 kDa) are required for the lysosomal hydrolysis of a variety of sphingolipids. Characterization of these four activator proteins, two of which were recently discovered, and their importance in human health and disease are reviewed in this article.

Publication Types:
- Review
- Review, Academic

PMID: 2001789 [PubMed - indexed for MEDLINE]

37: In Vitro Cell Dev Biol. 1988 Dec;24(12):1159-64. Related Articles, Links

Culture conditions found to minimize false positive diagnosis of lysosomal storage disorders.

Arnon J, Ornoy A, Bach G.

Dept. of Anatomy and Embryology, Hadassah Medical School; Jerusalem, Israel.

The effect of culture conditions on the ultrastructure and enzyme activities of cultured skin fibroblast cells relevant to the diagnosis of lysosomal storage disorders are reported. The parameters examined were: pH of the culture media, type of media, increasing cell passage, and day of harvest. Ultrastructural changes were defined in terms of the number of lysosome-like inclusion bodies per cell according to a method devised in our laboratory and proven reliable in the detection of affected individuals. Our biochemical results included determination of enzyme activities of beta-hexosaminidase, alpha-mannosidase, beta-glucuronidase-lysosomal enzymes, arylsulfatase C, a microsomal marker, and 5' nucleotidase, a plasma membrane marker. Our results indicate that the cellular ultrastructure is more sensitive than enzyme activity to changes in culture conditions. The resulting ultrastructural "artifacts" observed under certain conditions were severe enough to result in a mistaken diagnosis. Due to certain difficulties we had previously encountered in heterozygote cultures (for lysosomal storage disorders) of amniotic cells, we decided to examine heterozygote cultures of skin fibroblasts. From these (preliminary) studies it seems that an elevation in the pH over the physiologic levels in the culture media may help to define between normal individuals and affected heterozygotes. On the basis of our results, we recommend that to minimize false positive ultrastructural results for the diagnosis of lysosomal storage disorders, cultures be grown in minimal essential medium, the pH of the medium carefully monitored to remain below 7.4, examining the cultures not later than cell Passage 8 and no later than Day 10 after subculture.

PMID: 3209585 [PubMed - indexed for MEDLINE]

38: Am J Hum Genet. 1988 Feb;42(2):271-3. Related Articles, Links

Selection in favor of lysosomal storage disorders?

Zlotogora J, Zeigler M, Bach G.

Department of Human Genetics, Hadassah University Hospital, Jerusalem, Israel.

Four examples of Israeli communities or large families in which high consanguinity is common are presented, with two different lysosomal storage disorders within each community. In each of the four cases the stored substances share common chemical structure, despite the different lysosomal hydrolases involved in each disease. A similar phenomenon is known among the Ashkenazi Jews, in whom four of the most frequent hereditary disorders are lysosomal storage disorders, which are characterized by storage of sphingolipid derivatives. Similar findings are reported in the literature in other communities. We suggest that this phenomenon indicates a selection in favor of lysosomal storage disorders of similar nature in certain populations. The selection forces leading to this phenomenon have not been identified yet, and it has not yet been determined whether these forces are the same in the different communities presented here.

Publication Types:
- Case Reports

PMID: 3124612 [PubMed - indexed for MEDLINE]

☐ **39:** Trans Am Ophthalmol Soc. 1987;85:471-97. Related Articles, Links

The efficacy of conjunctival biopsy as a screening technique in lysosomal storage disorders.

Mazow ML.

Publication Types:
- Review
- Review of Reported Cases

PMID: 3328919 [PubMed - indexed for MEDLINE]

☐ **40:** Prenat Diagn. 1986 Sep-Oct;6(5):351-61. Related Articles, Links

Cultured amniotic fluid cells for prenatal diagnosis of lysosomal storage disorders: a methodological study.

Arnon J, Ornoy A, Bach G.

The influence of culture conditions on the ultrastructure and enzyme activities of amniotic fluid cells are reported. Morphological changes were determined as a function of the number of lysosomal-like inclusion bodies per cell, and these results correlated to the activity of beta-hexosaminidase, alpha-mannosidase, beta-glucuronidase, arylsulphatase C and 5' nucleotidase. The parameters examined were pH of the culture media, type of media, increasing cell passage and day of harvest. Our results indicate that enzyme activities are

less sensitive to changes in culture conditions as compared to ultrastructural changes. We therefore recommend that in order to obtain reliable ultrastructural results for the diagnosis of storage disorders, cultures should be grown in MEM as the culture medium, the pH of the medium carefully monitored to remain below pH 7.4, examining the cultures no later than the eighth cell passage and no later than the 10th day after subculture.

PMID: 3022278 [PubMed - indexed for MEDLINE]

☐ **41:** Hum Genet. 1986 Jul;73(3):214-7. Related Articles, Links

Free N-acetylneuraminic acid (NANA) storage disorders: evidence for defective NANA transport across the lysosomal membrane.

Mancini GM, Verheijen FW, Galjaard H.

To study the biochemical defect underlying N-acetylneuraminic acid (NANA) storage disorders (NSD), a tritium-labeled NANA-methylester was prepared and its metabolism was studied in normal and mutant human fibroblasts. The uptake of methylester, its conversion into free NANA, and the release of free NANA was studied in lysosome-enriched fractions. In three clinically different types of NSD accumulation of free NANA was observed and the half-life of this compound was significantly increased. Our observations indicate the existence of a transport system for NANA across the lysosomal membrane, which is deficient in all variants of NSD.

PMID: 3733077 [PubMed - indexed for MEDLINE]

☐ **42:** Lancet. 1985 Dec 7;2(8467):1296. Related Articles, Links

Pseudodeficiencies in lysosomal storage disorders.

Zlotogora J, Bach G.

Publication Types:
- Letter

PMID: 2866352 [PubMed - indexed for MEDLINE]

☐ **43:** Med J Aust. 1984 Feb 18;140(4):188-9. Related Articles, Links

Diagnosis of lysosomal storage disorders.

Kerr C.

PMID: 6694619 [PubMed - indexed for MEDLINE]

44: Virchows Arch B Cell Pathol Incl Mol Pathol. 1984;46(1-2):13-9. Related Articles, Links

Ito cells in lysosomal storage disorders. An ultrastructural study.

Elleder M.

An ultrastructural study was performed in a series of liver biopsies from patients with various lysosomal storage diseases to evaluate the extent of lysosomal hypertrophy and hyperplasia in Ito cells (ICs). In previous studies this has been considered to be absent or only rudimentary. Lysosomal storage was recognized by the presence of storage cytosomes surrounded by limiting membranes and by the appearance of their content which was identical to that in other hepatic storage lysosomes. Storage was found in sphingomyelinase deficiency (Niemann-Pick disease types A, B), in Wolman's disease, GM1 gangliosidosis, mucopolysaccharidosis and in multiple sulphatase deficiency. In type C Niemann-Pick disease it was virtually absent with the exception of cases with prominent hepatic symptomatology. Storage was of variable degree and was accompanied by a decrease in the physiological fat content (cytoplasmic lipid droplets). The degree to which ICs were affected correlated only with the extent to which nonspecific fibroblasts were involved in the specimens studied and thus seems to reflect storage in the fibroblastic population.

PMID: 6147922 [PubMed - indexed for MEDLINE]

45: Anal Biochem. 1980 Feb;102(1):213-9. Related Articles, Links

High-performance liquid chromatographic analysis of oligosaccharides and glycopeptides accumulating in lysosomal storage disorders.

Kin NM, Wolfe LS.

PMID: 6766687 [PubMed - indexed for MEDLINE]

46: Front Biol. 1979;48:49-130. Related Articles, Links

Drug-induced lysosomal storage disorders.

Lullmann-Rauch R.

Publication Types:
- Review

PMID: 387466 [PubMed - indexed for MEDLINE]

47: Birth Defects Orig Artic Ser. 1976;12(3):1-13. Related Articles, Links

Chemical diagnosis of inborn lysosomal storage disorders involving the eye.

Dawson G, Tsay GC.

Publication Types:
- Review

PMID: 821555 [PubMed - indexed for MEDLINE]

48: Arch Neurol. 1975 Sep;32(9):592-9. Related Articles, Links

Lysosomal storage disorders. Diagnosis by ultrastructural examination of skin biopsy specimens.

O'Brien JS, Bernett J, Veath ML, Paa D.

Fifteen patients with lysosomal storage diseases were studied. Diagnoses of their illnesses included infantile Gaucher disease; Krabbe disease; Niemann-Pick disease, type A; glycogen storage disease, type 3; Fabry disease, Jansky-Bielschowsky and Spielmeyer-Vogt types of amaurotic idiocy, GM1 gangliosidosis, type 1; Hurler disease; and Sanfilippo disease, types A and B. We carried out ultrastructural examinations of skin biopsy specimens that were taken to establish a cultured fibroblast line on each patient. We found diagnostic storage inclusions in all patients except those with infantile Gaucher disease, Krabbe disease, and Spielmeyer-Vogt disease, This technique can be carried out on a specimen obtained by a primary physician on an out-patient basis, thus avoiding major surgery.

PMID: 809024 [PubMed - indexed for MEDLINE]

NCBI PubMed National Library of Medicine NLM

Entrez PubMed Nucleotide Protein Genome Structure OMIM PMC Journals Boc

Search PubMed for "lysosomal storage" Go Clear

Limits Preview/Index History Clipboard Details

Field: **Title**, Limits: **Publication Date to 2001/10/28**

Display Summary Show: 100 Sort Send to Text

Items 1 - 100 of 338 Page 1 of 4 Ne

About Entrez

Text Version

Entrez PubMed
Overview
Help | FAQ
Tutorial
New/Noteworthy
E-Utilities

PubMed Services
Journals Database
MeSH Database
Single Citation Matcher
Batch Citation Matcher
Clinical Queries
LinkOut
Cubby

Related Resources
Order Documents
NLM Catalog
NLM Gateway
TOXNET
Consumer Health
Clinical Alerts
ClinicalTrials.gov
PubMed Central

1: Platt FM, Butters TD. Related Articles, Li

Substrate deprivation: a new therapeutic approach for the glycosphingolipid lysosomal storage diseases.
Expert Rev Mol Med. 2000 Feb 01;2000:1-17.
PMID: 14585134 [PubMed]

2: [No authors listed] Related Articles, Li

Lysosomal storage diseases. Fabry disease: new insights and future perspectives. Proceedings and abstracts of an international symposium. Seville, April 2001.
J Inherit Metab Dis. 2001;24 Suppl 2:1-185. No abstract available.
PMID: 11971228 [PubMed - indexed for MEDLINE]

3: Barker JE, Deveau S, Lessard M, Hamblen N, Vogler C, Levy B. Related Articles, Li

In utero fetal liver cell transplantation without toxic irradiation alleviates lysosomal storage in mice with mucopolysaccharidosis type VII.
Blood Cells Mol Dis. 2001 Sep-Oct;27(5):861-73.
PMID: 11783949 [PubMed - indexed for MEDLINE]

4: Vogler C, Barker J, Sands MS, Levy B, Galvin N, Sly WS. Related Articles, Li

Murine mucopolysaccharidosis VII: impact of therapies on the phenotype, clinical course, and pathology in a model of a lysosomal storage disease.
Pediatr Dev Pathol. 2001 Sep-Oct;4(5):421-33. Review.
PMID: 11779044 [PubMed - indexed for MEDLINE]

5: Bredehorn T, Clausen M, Duncker G, Lullmann-Rauch R. Related Articles, Li

Morphological and functional changes due to drug-induced lysosomal storag of sulphated glycosaminoglycans in the rat retina.
Graefes Arch Clin Exp Ophthalmol. 2001 Oct;239(10):788-93.
PMID: 11760042 [PubMed - indexed for MEDLINE]

6: Maire I. Related Articles, Li

Is genotype determination useful in predicting the clinical phenotype in lysosomal storage diseases?
J Inherit Metab Dis. 2001;24 Suppl 2:57-61; discussion 45-6. Review.
PMID: 11758680 [PubMed - indexed for MEDLINE]

7: Winchester B. Related Articles, Li

Are there useful biochemical markers of disease activity in lysosomal storag diseases?
J Inherit Metab Dis. 2001;24 Suppl 2:52-6; discussion 45-6. Review.

PMID: 11758679 [PubMed - indexed for MEDLINE]

8: Beck M. Related Articles, Li

Variable clinical presentation in lysosomal storage disorders.
J Inherit Metab Dis. 2001;24 Suppl 2:47-51; discussion 45-6. Review.
PMID: 11758678 [PubMed - indexed for MEDLINE]

9: Butters TD, Dwek RA, Platt FM. Related Articles, Li

Inhibition of glycosphingolipid biosynthesis: application to lysosomal storag disorders.
Chem Rev. 2000 Dec 13;100(12):4683-96. No abstract available.
PMID: 11749362 [PubMed - as supplied by publisher]

10: Barranger JM, Novelli EA. Related Articles, Li

Gene therapy for lysosomal storage disorders.
Expert Opin Biol Ther. 2001 Sep;1(5):857-67. Review.
PMID: 11728220 [PubMed - indexed for MEDLINE]

11: Estruch EJ, Hart SL, Kinnon C, Winchester BG. Related Articles, Li

Non-viral, integrin-mediated gene transfer into fibroblasts from patients wi lysosomal storage diseases.
J Gene Med. 2001 Sep-Oct;3(5):488-97.
PMID: 11601762 [PubMed - indexed for MEDLINE]

12: Kumamoto T. Related Articles, Li

[Amphiphilic cationic drug myopathy, drug-induced lysosomal storage lipidosis]
Ryoikibetsu Shokogun Shirizu. 2001;(36):263-6. Review. Japanese. No abstract available.
PMID: 11596386 [PubMed - indexed for MEDLINE]

13: Wisniewski KE, Kida E, Walus M, Wujek P, Kaczmarski W, Golabek AA. Related Articles, Li

Tripeptidyl-peptidase I in neuronal ceroid lipofuscinoses and other lysosom storage disorders.
Eur J Paediatr Neurol. 2001;5 Suppl A:73-9.
PMID: 11589013 [PubMed - indexed for MEDLINE]

14: Itoh M, Matsuda J, Suzuki O, Ogura A, Oshima A, Tai T, Suzuki Y, Takashima S. Related Articles, Li

Development of lysosomal storage in mice with targeted disruption of the beta-galactosidase gene: a model of human G(M1)-gangliosidosis.
Brain Dev. 2001 Oct;23(6):379-84.
PMID: 11578847 [PubMed - indexed for MEDLINE]

15: Wraith JE. Related Articles, Li

Advances in the treatment of lysosomal storage disease.
Dev Med Child Neurol. 2001 Sep;43(9):639-46. Review. No abstract available.
PMID: 11570635 [PubMed - indexed for MEDLINE]

16: Weibel TD, Brady RO. Related Articles, Li

Systematic approach to the diagnosis of lysosomal storage disorders.
Ment Retard Dev Disabil Res Rev. 2001;7(3):190-9. Review.
PMID: 11553935 [PubMed - indexed for MEDLINE]

17: Yew NS, Cheng SH. Related Articles, Li

Gene therapy for lysosomal storage disorders.
Curr Opin Mol Ther. 2001 Aug;3(4):399-406. Review.
PMID: 11525564 [PubMed - indexed for MEDLINE]

18: Platt FM, Jeyakumar M, Andersson U, Priestman DA, Dwek RA, Butters TD, Cox TM, Lachmann RH, Hollak C, Aerts JM, Van Weely S, Hrebicek M, Moyses C, Gow I, Elstein D, Zimran A. Related Articles, Li

Inhibition of substrate synthesis as a strategy for glycolipid lysosomal stora disease therapy.
J Inherit Metab Dis. 2001 Apr;24(2):275-90. Review.
PMID: 11405346 [PubMed - indexed for MEDLINE]

19: Ranieri E, Gerace RL, Ravenscroft EM, Hopwood JJ, Meikle PJ. Related Articles, Li

Pilot neonatal screening program for lysosomal storage disorders, using lamp-1.
Southeast Asian J Trop Med Public Health. 1999;30 Suppl 2:111-3.
PMID: 11400745 [PubMed - indexed for MEDLINE]

20: Meikle PJ, Ranieri E, Ravenscroft EM, Hua CT, Brooks DA, Hopwood JJ. Related Articles, Li

Newborn screening for lysosomal storage disorders.
Southeast Asian J Trop Med Public Health. 1999;30 Suppl 2:104-10.
PMID: 11400743 [PubMed - indexed for MEDLINE]

21: Tylki-Szymanska A, Czartoryska B, Lugowska A, Gorska D. Related Articles, Li

The prevalence and diagnosis of lysosomal storage diseases in Poland.
Eur J Pediatr. 2001 Apr;160(4):261-2. No abstract available.
PMID: 11317653 [PubMed - indexed for MEDLINE]

22: Poenaru L. Related Articles, Li

From gene transfer to gene therapy in lysosomal storage diseases affecting the central nervous system.
Ann Med. 2001 Feb;33(1):28-36. Review.
PMID: 11310936 [PubMed - indexed for MEDLINE]

23: Ryder SJ, Simmons MM. Related Articles, Li

A lysosomal storage disease of Romney sheep that resembles human type 2 GM1 gangliosidosis.
Acta Neuropathol (Berl). 2001 Mar;101(3):225-8.
PMID: 11307621 [PubMed - indexed for MEDLINE]

24: Kaye EM. Related Articles, Li

Lysosomal Storage Diseases.
Curr Treat Options Neurol. 2001 May;3(3):249-256.
PMID: 11282040 [PubMed - as supplied by publisher]

25: Guy R, Forsyth JM, Cooper A, Morton RE. Related Articles, Li

Co-existence of lysosomal storage diseases in a consanguineous family.
Child Care Health Dev. 2001 Mar;27(2):173-81.
PMID: 11251615 [PubMed - indexed for MEDLINE]

26: Kosuga M, Sasaki K, Tanabe A, Li XK, Okawa H, Ogino I, Okuda O, Arai H, Sakuragawa N, Kamata Y, Azuma N, Suzuki S, Yamada M, Okuyama T. Related Articles, Li

Engraftment of genetically engineered amniotic epithelial cells corrects

lysosomal storage in multiple areas of the brain in mucopolysaccharidosis type VII mice.
Mol Ther. 2001 Feb;3(2):139-48.
PMID: 11237670 [PubMed - indexed for MEDLINE]

27: Ward DM, Griffiths GM, Stinchcombe JC, Kaplan J. Related Articles, Li

Analysis of the lysosomal storage disease Chediak-Higashi syndrome.
Traffic. 2000 Nov;1(11):816-22. Review.
PMID: 11208072 [PubMed - indexed for MEDLINE]

28: Wolfe JH, Sands MS, Harel N, Weil MA, Parente MK, Polesky AC, Reilly JJ, Hasson C, Weimelt S, Haskins ME. Related Articles, Li

Gene transfer of low levels of beta-glucuronidase corrects hepatic lysosom storage in a large animal model of mucopolysaccharidosis VII.
Mol Ther. 2000 Dec;2(6):552-61.
PMID: 11124056 [PubMed - indexed for MEDLINE]

29: Warren CD, Alroy J. Related Articles, Li

Morphological, biochemical and molecular biology approaches for the diagnosis of lysosomal storage diseases.
J Vet Diagn Invest. 2000 Nov;12(6):483-96. Review. No abstract available.
PMID: 11108447 [PubMed - indexed for MEDLINE]

30: Walch E, Schmidt M, Brenner RE, Emons D, Dame C, Pontz B, Wiestler OD, Bartmann P. Related Articles, Li

Yunis-Varon syndrome: evidence for a lysosomal storage disease.
Am J Med Genet. 2000 Nov 13;95(2):157-60.
PMID: 11078567 [PubMed - indexed for MEDLINE]

31: Simons K, Gruenberg J. Related Articles, Li

Jamming the endosomal system: lipid rafts and lysosomal storage diseases.
Trends Cell Biol. 2000 Nov;10(11):459-62.
PMID: 11050411 [PubMed - indexed for MEDLINE]

32: Ross CJ, Bastedo L, Maier SA, Sands MS, Chang PL. Related Articles, Li

Treatment of a lysosomal storage disease, mucopolysaccharidosis VII, with microencapsulated recombinant cells.
Hum Gene Ther. 2000 Oct 10;11(15):2117-27.
PMID: 11044913 [PubMed - indexed for MEDLINE]

33: Ohlemiller KK, Vogler CA, Roberts M, Galvin N, Sands MS. Related Articles, Li

Retinal function is improved in a murine model of a lysosomal storage disease following bone marrow transplantation.
Exp Eye Res. 2000 Nov;71(5):469-81.
PMID: 11040082 [PubMed - indexed for MEDLINE]

34: Schuchman EH. Related Articles, Li

Hematopoietic stem cell gene therapy for Niemann-Pick disease and other lysosomal storage diseases.
Chem Phys Lipids. 1999 Nov;102(1-2):179-88. Review.
PMID: 11001572 [PubMed - indexed for MEDLINE]

35: Koike M, Nakanishi H, Saftig P, Ezaki J, Isahara K, Ohsawa Y, Schulz-Schaeffer W, Watanabe T, Waguri S, Kametaka S, Shibata M, Yamamoto K, Kominami E, Peters C, von Figura K, Uchiyama Y. Related Articles, Li

Cathepsin D deficiency induces lysosomal storage with ceroid lipofuscin ir mouse CNS neurons.
J Neurosci. 2000 Sep 15;20(18):6898-906.
PMID: 10995834 [PubMed - indexed for MEDLINE]

36: Gao C, Sands MS, Haskins ME, Ponder KP. Related Articles, Li

Delivery of a retroviral vector expressing human beta-glucuronidase to the liver and spleen decreases lysosomal storage in mucopolysaccharidosis VII mice.
Mol Ther. 2000 Sep;2(3):233-44.
PMID: 10985954 [PubMed - indexed for MEDLINE]

37: Umapathysivam K, Whittle AM, Ranieri E, Bindloss C, Ravenscroft EM, van Diggelen OP, Hopwood JJ, Meikle PJ. Related Articles, Li

Determination of acid alpha-glucosidase protein: evaluation as a screening marker for Pompe disease and other lysosomal storage disorders.
Clin Chem. 2000 Sep;46(9):1318-25.
PMID: 10973860 [PubMed - indexed for MEDLINE]

38: Ioannou YA. Related Articles, Li

Gene therapy for lysosomal storage disorders with neuropathology.
J Am Soc Nephrol. 2000 Aug;11(8):1542-7. Review. No abstract available.
PMID: 10906169 [PubMed - indexed for MEDLINE]

39: Eto Y, Ohashi T. Related Articles, Li

Gene therapy/cell therapy for lysosomal storage disease.
J Inherit Metab Dis. 2000 May;23(3):293-8.
PMID: 10863945 [PubMed - indexed for MEDLINE]

40: Tyynela J, Sohar I, Sleat DE, Gin RM, Donnelly RJ, Baumann M, Haltia M, Lobel P. Related Articles, Li

A mutation in the ovine cathepsin D gene causes a congenital lysosomal storage disease with profound neurodegeneration.
EMBO J. 2000 Jun 15;19(12):2786-92.
PMID: 10856224 [PubMed - indexed for MEDLINE]

41: Daly TM, Lorenz RG, Sands MS. Related Articles, Li

Abnormal immune function in vivo in a murine model of lysosomal storage disease.
Pediatr Res. 2000 Jun;47(6):757-62.
PMID: 10832733 [PubMed - indexed for MEDLINE]

42: Ohashi T, Yokoo T, Iizuka S, Kobayashi H, Sly WS, Eto Y. Related Articles, Li

Reduction of lysosomal storage in murine mucopolysaccharidosis type VII by transplantation of normal and genetically modified macrophages.
Blood. 2000 Jun 1;95(11):3631-3.
PMID: 10828055 [PubMed - indexed for MEDLINE]

43: Winchester B, Vellodi A, Young E. Related Articles, Li

The molecular basis of lysosomal storage diseases and their treatment.
Biochem Soc Trans. 2000 Feb;28(2):150-4. Review.
PMID: 10816117 [PubMed - indexed for MEDLINE]

44: Grewal RP, Yu KT, Barton NW, Parker RI, Bisceglie AM. Related Articles, Li

Liver biopsies in patients with lysosomal storage disease: experience with

effective sedation.
Indian J Pediatr. 1997 Nov-Dec;64(6):887-91.
PMID: 10771935 [PubMed - indexed for MEDLINE]

45: Sferra TJ, Qu G, McNeely D, Rennard R, Clark KR, Lo WD, Johnson PR. Related Articles, Li

Recombinant adeno-associated virus-mediated correction of lysosomal storage within the central nervous system of the adult mucopolysaccharido: type VII mouse.
Hum Gene Ther. 2000 Mar 1;11(4):507-19.
PMID: 10724030 [PubMed - indexed for MEDLINE]

46: Driemeier D, Colodel EM, Gimeno EJ, Barros SS. Related Articles, Li

Lysosomal storage disease caused by Sida carpinifolia poisoning in goats.
Vet Pathol. 2000 Mar;37(2):153-9.
PMID: 10714644 [PubMed - indexed for MEDLINE]

47: Chang MH, Bindloss CA, Grabowski GA, Qi X, Winchester B, Hopwood JJ, Meikle PJ. Related Articles, Li

Saposins A, B, C, and D in plasma of patients with lysosomal storage disorders.
Clin Chem. 2000 Feb;46(2):167-74.
PMID: 10657372 [PubMed - indexed for MEDLINE]

48: Stone DL, Sidransky E. Related Articles, Li

Hydrops fetalis: lysosomal storage disorders in extremis.
Adv Pediatr. 1999;46:409-40. Review.
PMID: 10645471 [PubMed - indexed for MEDLINE]

49: Isaac EL, Karageorgos LE, Brooks DA, Hopwood JJ, Meikle PJ. Related Articles, Li

Regulation of the lysosome-associated membrane protein in a sucrose mod of lysosomal storage.
Exp Cell Res. 2000 Feb 1;254(2):204-9.
PMID: 10640418 [PubMed - indexed for MEDLINE]

50: Skorupa AF, Fisher KJ, Wilson JM, Parente MK, Wolfe JH. Related Articles, Li

Sustained production of beta-glucuronidase from localized sites after AAV vector gene transfer results in widespread distribution of enzyme and rever of lysosomal storage lesions in a large volume of brain in mucopolysaccharidosis VII mice.
Exp Neurol. 1999 Nov;160(1):17-27.
PMID: 10630187 [PubMed - indexed for MEDLINE]

51: Crawley AC, Jones MZ, Bonning LE, Finnie JW, Hopwood JJ. Related Articles, Li

Alpha-mannosidosis in the guinea pig: a new animal model for lysosomal storage disorders.
Pediatr Res. 1999 Nov;46(5):501-9.
PMID: 10541310 [PubMed - indexed for MEDLINE]

52: Michalski JC, Klein A. Related Articles, Li

Glycoprotein lysosomal storage disorders: alpha- and beta-mannosidosis, fucosidosis and alpha-N-acetylgalactosaminidase deficiency.
Biochim Biophys Acta. 1999 Oct 8;1455(2-3):69-84. Review.
PMID: 10571005 [PubMed - indexed for MEDLINE]

53: Brooks DA. Related Articles, Li

Immune response to enzyme replacement therapy in lysosomal storage disorder patients and animal models.
Mol Genet Metab. 1999 Oct;68(2):268-75. Review.
PMID: 10527678 [PubMed - indexed for MEDLINE]

54: Groener JE, de Graaf FL, Poorthuis BJ, Kanhai HH. Related Articles, Li

Prenatal diagnosis of lysosomal storage diseases using fetal blood.
Prenat Diagn. 1999 Oct;19(10):930-3.
PMID: 10521818 [PubMed - indexed for MEDLINE]

55: Folkerth RD. Related Articles, Li

Abnormalities of developing white matter in lysosomal storage diseases.
J Neuropathol Exp Neurol. 1999 Sep;58(9):887-902. Review.
PMID: 10499433 [PubMed - indexed for MEDLINE]

56: Poorthuis BJ, Wevers RA, Kleijer WJ, Groener JE, de Jong JG, van Weely S, Niezen-Koning KE, van Diggelen OP. Related Articles, Li

The frequency of lysosomal storage diseases in The Netherlands.
Hum Genet. 1999 Jul-Aug;105(1-2):151-6.
PMID: 10480370 [PubMed - indexed for MEDLINE]

57: Elliger SS, Elliger CA, Aguilar CP, Raju NR, Watson GL. Related Articles, Li

Elimination of lysosomal storage in brains of MPS VII mice treated by intrathecal administration of an adeno-associated virus vector.
Gene Ther. 1999 Jun;6(6):1175-8.
PMID: 10455422 [PubMed - indexed for MEDLINE]

58: Ziegler RJ, Yew NS, Li C, Cherry M, Berthelette P, Romanczuk H, Ioannou YA, Zeidner KM, Desnick RJ, Cheng SH. Related Articles, Li

Correction of enzymatic and lysosomal storage defects in Fabry mice by adenovirus-mediated gene transfer.
Hum Gene Ther. 1999 Jul 1;10(10):1667-82.
PMID: 10428212 [PubMed - indexed for MEDLINE]

59: Bennett MJ, Hofmann SL. Related Articles, Li

The neuronal ceroid-lipofuscinoses (Batten disease): a new class of lysosomal storage diseases.
J Inherit Metab Dis. 1999 Jun;22(4):535-44. Review.
PMID: 10407785 [PubMed - indexed for MEDLINE]

60: de Balogh KK, Dimande AP, van der Lugt JJ, Molyneux RJ, Naude TW, Welman WG. Related Articles, Li

A lysosomal storage disease induced by Ipomoea carnea in goats in Mozambique.
J Vet Diagn Invest. 1999 May;11(3):266-73.
PMID: 10353359 [PubMed - indexed for MEDLINE]

61: Sleat DE, Gin RM, Sohar I, Wisniewski K, Sklower-Brooks S, Pullarkat RK, Palmer DN, Lerner TJ, Boustany RM, Uldall P, Siakotos AN, Donnelly RJ, Lobel P. Related Articles, Li

Mutational analysis of the defective protease in classic late-infantile neuron ceroid lipofuscinosis, a neurodegenerative lysosomal storage disorder.
Am J Hum Genet. 1999 Jun;64(6):1511-23.
PMID: 10330339 [PubMed - indexed for MEDLINE]

62: Barranger JA, Rice EO, Swaney WP. Related Articles, Li

Gene transfer approaches to the lysosomal storage disorders.
Neurochem Res. 1999 Apr;24(4):601-15. Review.
PMID: 10227692 [PubMed - indexed for MEDLINE]

63: Stein CS, Ghodsi A, Derksen T, Davidson BL. Related Articles, Li

Systemic and central nervous system correction of lysosomal storage in mucopolysaccharidosis type VII mice.
J Virol. 1999 Apr;73(4):3424-9.
PMID: 10074197 [PubMed - indexed for MEDLINE]

64: Daly TM, Vogler C, Levy B, Haskins ME, Sands MS. Related Articles, Li

Neonatal gene transfer leads to widespread correction of pathology in a murine model of lysosomal storage disease.
Proc Natl Acad Sci U S A. 1999 Mar 2;96(5):2296-300.
PMID: 10051635 [PubMed - indexed for MEDLINE]

65: Watson GL, Sayles JN, Chen C, Elliger SS, Elliger CA, Raju NR, Kurtzman GJ, Podsakoff GM. Related Articles, Li

Treatment of lysosomal storage disease in MPS VII mice using a recombinant adeno-associated virus.
Gene Ther. 1998 Dec;5(12):1642-9.
PMID: 10023443 [PubMed - indexed for MEDLINE]

66: Peltola M, Kyttala A, Heinonen O, Rapola J, Paunio T, Revah F, Peltonen L, Jalanko A. Related Articles, Li

Adenovirus-mediated gene transfer results in decreased lysosomal storage i brain and total correction in liver of aspartylglucosaminuria (AGU) mouse.
Gene Ther. 1998 Oct;5(10):1314-21.
PMID: 9930336 [PubMed - indexed for MEDLINE]

67: Meikle PJ, Hopwood JJ, Clague AE, Carey WF. Related Articles, Li

Prevalence of lysosomal storage disorders.
JAMA. 1999 Jan 20;281(3):249-54.
PMID: 9918480 [PubMed - indexed for MEDLINE]

68: Diettrich O, Mills K, Johnson AW, Hasilik A, Winchester BG. Related Articles, Li

Application of magnetic chromatography to the isolation of lysosomes fron fibroblasts of patients with lysosomal storage disorders.
FEBS Lett. 1998 Dec 28;441(3):369-72.
PMID: 9891973 [PubMed - indexed for MEDLINE]

69: Platt FM, Butters TD. Related Articles, Li

New therapeutic prospects for the glycosphingolipid lysosomal storage diseases.
Biochem Pharmacol. 1998 Aug 15;56(4):421-30. Review.
PMID: 9763217 [PubMed - indexed for MEDLINE]

70: Hua CT, Hopwood JJ, Carlsson SR, Harris RJ, Meikle PJ. Related Articles, Li

Evaluation of the lysosome-associated membrane protein LAMP-2 as a marker for lysosomal storage disorders.
Clin Chem. 1998 Oct;44(10):2094-102.
PMID: 9761240 [PubMed - indexed for MEDLINE]

Rottier RJ, Hahn CN, Mann LW, del Pilar Martin M, Smeyne RJ, Related Articles, Li

71: Suzuki K, d'Azzo A.

Lack of PPCA expression only partially coincides with lysosomal storage i galactosialidosis mice: indirect evidence for spatial requirement of the catalytic rather than the protective function of PPCA.
Hum Mol Genet. 1998 Oct;7(11):1787-94.
PMID: 9736781 [PubMed - indexed for MEDLINE]

72: Vogler C, Sands MS, Galvin N, Levy B, Thorpe C, Barker J, Sly WS. Related Articles, Li

Murine mucopolysaccharidosis type VII: the impact of therapies on the clinical course and pathology in a murine model of lysosomal storage disease.
J Inherit Metab Dis. 1998 Aug;21(5):575-86. Review.
PMID: 9728337 [PubMed - indexed for MEDLINE]

73: Hoogerbrugge PM, Valerio D. Related Articles, Li

Bone marrow transplantation and gene therapy for lysosomal storage diseases.
Bone Marrow Transplant. 1998 Apr;21 Suppl 2:S34-6. Review.
PMID: 9630322 [PubMed - indexed for MEDLINE]

74: Maaswinkel-Mooij PD, Poorthuis BJ, Hoogerbrugge PM, Brouwer OF, Vossen JM. Related Articles, Li

[Allogeneic bone marrow transplantation in the treatment of (lysosomal) storage diseases]
Ned Tijdschr Geneeskd. 1998 Jan 24;142(4):169-74. Review. Dutch.
PMID: 9557021 [PubMed - indexed for MEDLINE]

75: Walkley SU. Related Articles, Li

Cellular pathology of lysosomal storage disorders.
Brain Pathol. 1998 Jan;8(1):175-93. Review.
PMID: 9458175 [PubMed - indexed for MEDLINE]

76: Lake BD, Young EP, Winchester BG. Related Articles, Li

Prenatal diagnosis of lysosomal storage diseases.
Brain Pathol. 1998 Jan;8(1):133-49.
PMID: 9458172 [PubMed - indexed for MEDLINE]

77: Bouvier R, Maire I. Related Articles, Li

[Diagnosis of lysosomal storage diseases with fetal presentation]
Ann Pathol. 1997 Sep;17(4):277-80. Review. French.
PMID: 9409888 [PubMed - indexed for MEDLINE]

78: Jolly RD, Walkley SU. Related Articles, Li

Lysosomal storage diseases of animals: an essay in comparative pathology.
Vet Pathol. 1997 Nov;34(6):527-48. Review.
PMID: 9396134 [PubMed - indexed for MEDLINE]

79: Kieseier BC, Wisniewski KE, Goebel HH. Related Articles, Li

The monocyte-macrophage system is affected in lysosomal storage disease an immunoelectron microscopic study.
Acta Neuropathol (Berl). 1997 Oct;94(4):359-62.
PMID: 9341937 [PubMed - indexed for MEDLINE]

80: Fischer J, Lullmann H, Lullmann-Rauch R. Related Articles, Li

Drug-induced lysosomal storage of sulphated glycosaminoglycans.
Gen Pharmacol. 1996 Dec;27(8):1317-24. Review.
PMID: 9304401 [PubMed - indexed for MEDLINE]

81: Meikle PJ, Brooks DA, Ravenscroft EM, Yan M, Williams RE, Jaunzems AE, Chataway TK, Karageorgos LE, Davey RC, Boulter CD, Carlsson SR, Hopwood JJ. Related Articles, Li

Diagnosis of lysosomal storage disorders: evaluation of lysosome-associate membrane protein LAMP-1 as a diagnostic marker.
Clin Chem. 1997 Aug;43(8 Pt 1):1325-35.
PMID: 9267309 [PubMed - indexed for MEDLINE]

82: Saftig P, Hartmann D, Lullmann-Rauch R, Wolff J, Evers M, Koster A, Hetman M, von Figura K, Peters C. Related Articles, Li

Mice deficient in lysosomal acid phosphatase develop lysosomal storage in the kidney and central nervous system.
J Biol Chem. 1997 Jul 25;272(30):18628-35.
PMID: 9228031 [PubMed - indexed for MEDLINE]

83: Katz ML, Siakotos AN, Gao Q, Freiha B, Chin DT. Related Articles, Li

Late-infantile ceroid-lipofuscinosis: lysine methylation of mitochondrial A' synthase subunit c from lysosomal storage bodies.
Biochim Biophys Acta. 1997 Jul 10;1361(1):66-74.
PMID: 9247091 [PubMed - indexed for MEDLINE]

84: Karageorgos LE, Isaac EL, Brooks DA, Ravenscroft EM, Davey R, Hopwood JJ, Meikle PJ. Related Articles, Li

Lysosomal biogenesis in lysosomal storage disorders.
Exp Cell Res. 1997 Jul 10;234(1):85-97.
PMID: 9223373 [PubMed - indexed for MEDLINE]

85: Bennett MJ, Boriack RL, Boustany RM. Related Articles, Li

Polyunsaturated fatty acids reverse the lysosomal storage and accumulatior of subunit 9 of mitochondrial F1F0-ATP synthase in cultured lymphoblasts from patients with Batten disease.
J Inherit Metab Dis. 1997 Jul;20(3):457-60. No abstract available.
PMID: 9266381 [PubMed - indexed for MEDLINE]

86: Taylor RM, Wolfe JH. Related Articles, Li

Decreased lysosomal storage in the adult MPS VII mouse brain in the vicinity of grafts of retroviral vector-corrected fibroblasts secreting high levels of beta-glucuronidase.
Nat Med. 1997 Jul;3(7):771-4.
PMID: 9212105 [PubMed - indexed for MEDLINE]

87: Sly WS, Vogler C. Related Articles, Li

Gene therapy for lysosomal storage disease: a no-brainer? Transplants of fibroblasts secreting high levels of beta-glucuronidase decrease lesions in tl brains of mice with Sly syndrome, a lysosomal storage disease.
Nat Med. 1997 Jul;3(7):719-20. No abstract available.
PMID: 9212092 [PubMed - indexed for MEDLINE]

88: Platt FM, Neises GR, Reinkensmeier G, Townsend MJ, Perry VH, Proia RL, Winchester B, Dwek RA, Butters TD. Related Articles, Li

Prevention of lysosomal storage in Tay-Sachs mice treated with N-

butyldeoxynojirimycin.
Science. 1997 Apr 18;276(5311):428-31.
PMID: 9103204 [PubMed - indexed for MEDLINE]

89: Kattner E, Schafer A, Harzer K. Related Articles, Li

Hydrops fetalis: manifestation in lysosomal storage diseases including Fart disease.
Eur J Pediatr. 1997 Apr;156(4):292-5. Review.
PMID: 9128814 [PubMed - indexed for MEDLINE]

90: Krasnopolskaya XD, Mirenburg TV, Akhunov VS, Voskoboeva EY. Related Articles, Li

Postnatal and prenatal diagnosis of lysosomal storage diseases in the forme Soviet Union.
Wien Klin Wochenschr. 1997 Feb 14;109(3):74-80.
PMID: 9060146 [PubMed - indexed for MEDLINE]

91: Ueda Y, Tomita S. Related Articles, Li

[Lysosomal storage disease]
Ryoikibetsu Shokogun Shirizu. 1997;(17 Pt 2):284-7. Review. Japanese. No abstract available.
PMID: 9277918 [PubMed - indexed for MEDLINE]

92: Poenaru L. Related Articles, Li

Particularities of lysosomal storage diseases gene therapy.
Gene Ther. 1996 Dec;3(12):1039-41. No abstract available.
PMID: 8986430 [PubMed - indexed for MEDLINE]

93: Lipman RD, Donohue LR, Hoppe P, Bronson RT. Related Articles, Li

Evidence that lysosomal storage of proteolipids is a cell autonomous proce: in the motor neuron degeneration (mnd) mouse, a model of neuronal ceroid lipofuscinosis.
Neurosci Lett. 1996 Nov 22;219(2):111-4.
PMID: 8971792 [PubMed - indexed for MEDLINE]

94: Lullmann-Rauch R, von Witzendorff B. Related Articles, Li

Lysosomal storage of sulphated glycosaminoglycans induced by dicationic amphiphilic drug molecules: significance of the central planar ring system.
Pharmacol Toxicol. 1996 Sep;79(3):109-13.
PMID: 8884867 [PubMed - indexed for MEDLINE]

95: Raben N, Nichols RC, Martiniuk F, Plotz PH. Related Articles, Li

A model of mRNA splicing in adult lysosomal storage disease (glycogenos type II).
Hum Mol Genet. 1996 Jul;5(7):995-1000.
PMID: 8817337 [PubMed - indexed for MEDLINE]

96: Prasad A, Kaye EM, Alroy J. Related Articles, Li

Electron microscopic examination of skin biopsy as a cost-effective tool in the diagnosis of lysosomal storage diseases.
J Child Neurol. 1996 Jul;11(4):301-8.
PMID: 8807420 [PubMed - indexed for MEDLINE]

97: Lullmann-Rauch R, Pods R, von Witzendorff B. Related Articles, Li

The antimalarials quinacrine and chloroquine induce weak lysosomal stora;

of sulphated glycosaminoglycans in cell culture and in vivo.
Toxicology. 1996 Jun 17;110(1-3):27-37.
PMID: 8658557 [PubMed - indexed for MEDLINE]

98: Vogler C, Sands MS, Levy B, Galvin N, Birkenmeier EH, Sly WS. Related Articles, Li

Enzyme replacement with recombinant beta-glucuronidase in murine mucopolysaccharidosis type VII: impact of therapy during the first six wee of life on subsequent lysosomal storage, growth, and survival.
Pediatr Res. 1996 Jun;39(6):1050-4.
PMID: 8725268 [PubMed - indexed for MEDLINE]

99: Kim DY, Cho DY, Taylor HW. Related Articles, Li

Lysosomal storage disease in an emu (Dromaius novaehollandiae).
Vet Pathol. 1996 May;33(3):365-6.
PMID: 8740717 [PubMed - indexed for MEDLINE]

100: Piraud M, Froissart R, Mandon G, Bernard A, Maire I. Related Articles, Li

Amniotic fluid for screening of lysosomal storage diseases presenting in utero (mainly as non-immune hydrops fetalis).
Clin Chim Acta. 1996 Apr 30;248(2):143-55.
PMID: 8740579 [PubMed - indexed for MEDLINE]

Items 1 - 100 of 338 Page 1 of 4 Ne

Display Summary Show: 100 Sort Send to Text

Write to the Help Desk
NCBI | NLM | NIH
Department of Health & Human Services
Privacy Statement | Freedom of Information Act | Disclaimer

Dec 13 2004 14:18:14

NCBI

HOME | SEARCH | SITE MAP

Entrez, The Life Sciences Search Engine

PubMed | Entrez | Human Genome | GenBank | Map Viewer | BLAS

Search across databases "lysosomal storage disease" GO CLEAR Help

580	**PubMed:** biomedical literature citations and abstracts		8	**Books:** online books	
71	**PubMed Central:** free, full text journal articles		25	**OMIM:** online Mendelian Inheritance in Man	
			none	**Site Search:** NCBI web and FTP sites	
25	**Nucleotide:** sequence database (GenBank)		1	**UniGene:** gene-oriented clusters of transcript sequences	
40	**Protein:** sequence database		1	**CDD:** conserved protein domain database	
none	**Genome:** whole genome sequences		none	**3D Domains:** domains from Entrez Structure	
none	**Structure:** three-dimensional macromolecular structures		none	**UniSTS:** markers and mapping data	
none	**Taxonomy:** organisms in GenBank		none	**PopSet:** population study data sets	
none	**SNP:** single nucleotide polymorphism		none	**GEO Profiles:** expression and molecular abundance profiles	
6	**Gene:** gene-centered information		none	**GEO DataSets:** experimental sets of GEO data	
5	**HomoloGene:** eukaryotic homology groups		1665	**Cancer Chromosomes:** cytogenetic databases	
none	**PubChem Compound:** small molecule chemical structures		none	**PubChem BioAssay:** bioactivity screens of chemical substances	
none	**PubChem Substance:** chemical substances screened for bioactivity		none	**GENSAT:** gene expression atlas of mouse central nervous system	
88	**Journals:** detailed information *about* the journals indexed in PubMed and other Entrez databases		8	**MeSH:** detailed information about NLM's controlled vocabulary	
1	**NLM Catalog:** catalog of books, journals, and audiovisuals in the NLM collections				

- Result counts displayed in gray indicate one or more terms not found

| Counts in XML | Entrez Utilities | Disclaimer | Privacy statement | Accessibi

ALDURAZYME® (LARONIDASE)

Solution for Intravenous Infusion Only

DESCRIPTION

ALDURAZYME® (laronidase) is a polymorphic variant of the human enzyme, α-L-iduronidase that is produced by recombinant DNA technology in a Chinese hamster ovary cell line. α-L-iduronidase (glycosaminoglycan α-L-iduronohydrolase, EC 3.2.1.76) is a lysosomal hydrolase that catalyses the hydrolysis of terminal α-L-iduronic acid residues of dermatan sulfate and heparan sulfate.

Laronidase is a glycoprotein with a molecular weight of approximately 83 kD. The predicted amino acid sequence of the recombinant form, as well as the nucleotide sequence that encodes it, are identical to a polymorphic form of human α-L-iduronidase. The recombinant protein is comprised of 628 amino acids after cleavage of the N-terminus and contains 6 N-linked oligosaccharide modification sites. Two oligosaccharide chains terminate in mannose-6-phosphate sugars. ALDURAZYME has a specific activity of approximately 172 U/mg.

ALDURAZYME, for intravenous infusion, is supplied as a sterile, nonpyrogenic, colorless to pale yellow, clear to slightly opalescent solution that must be diluted prior to administration in 0.9% Sodium Chloride Injection, USP, containing 0.1% Albumin (Human). The solution in each vial contains a nominal laronidase concentration of 0.58 mg/mL and a pH of approximately 5.5. The extractable volume of 5.0 mL from each vial provides 2.9 mg laronidase, 43.9 mg sodium chloride, 63.5 mg sodium phosphate monobasic monohydrate, 10.7 mg sodium phosphate dibasic heptahydrate, and 0.05 mg polysorbate 80. ALDURAZYME does not contain preservatives; vials are for single use only.

CLINICAL PHARMACOLOGY

Mechanism of Action

Mucopolysaccharide storage disorders are caused by the deficiency of specific lysosomal enzymes required for the catabolism of glycosaminoglycans (GAG). Mucopolysaccharidosis I (MPS I) is characterized by the deficiency of α-L-iduronidase, a lysosomal hydrolase which catalyses the hydrolysis of terminal α-L-iduronic acid residues

of dermatan sulfate and heparan sulfate. Reduced or absent α-L-iduronidase activity results in the accumulation of the GAG substrates, dermatan sulfate and heparan sulfate, throughout the body and leads to widespread cellular, tissue, and organ dysfunction.

The rationale of ALDURAZYME therapy in MPS I is to provide exogenous enzyme for uptake into lysosomes and increase the catabolism of GAG. ALDURAZYME uptake by cells into lysosomes is most likely mediated by the mannose-6-phosphate-terminated oligosaccharide chains of laronidase binding to specific mannose-6-phosphate receptors.

Because many proteins in the blood are restricted from entry into the central nervous system by the blood brain barrier, effects of intravenously administered ALDURAZYME on cells within the central nervous system (CNS) cannot be inferred from activity in sites outside the CNS. The ability of ALDURAZYME to cross the blood brain barrier has not been evaluated in animal models or in clinical trials.

Pharmacokinetics

The pharmacokinetics of laronidase were evaluated in 12 patients with MPS I who received 0.58 mg/kg of ALDURAZYME as a 4 hour infusion. After the 1st, 12th and 26th weekly infusions, the mean maximum plasma concentrations (C_{max}) ranged from 1.2 to 1.7 mcg/mL for the 3 time points. The mean area under the plasma concentration-time curve (AUC_{∞}) ranged from 4.5 to 6.9 mcg•hour/mL. The mean volume of distribution (V_z) ranged from 0.24 to 0.6 L/kg. Mean plasma clearance (CL) ranged from 1.7 to 2.7 mL/min/kg, and the mean elimination half-life ($t_{1/2}$) ranged from 1.5 to 3.6 hours.

Effects of Antibodies

Most patients who received once-weekly infusions of ALDURAZYME developed antibodies to laronidase by week 12. Between weeks 1 and 12, increases in plasma clearance of laronidase were observed in some patients which appeared to be proportional to the antibody titer. At week 26, plasma clearance of laronidase was comparable to that at week 1, in spite of the continued and, in some cases, increased titers of antibodies.

CLINICAL STUDIES

ALDURAZYME was studied in a randomized, placebo-controlled clinical trial of 45 MPS I patients of whom 1 patient was clinically assessed as having the Hurler form, 37 Hurler-Scheie, and 7 Scheie. All patients had a baseline forced vital capacity (FVC) less than or

equal to 77% of predicted. Patients received ALDURAZYME at 0.58 mg/kg or placebo once-weekly for 26 weeks. All patients were treated with antipyretics and antihistamines prior to each infusion.

The primary efficacy outcome assessments were FVC and distance walked in 6 minutes (6-minute walk test, 6MWT). After 26 weeks, patients treated with ALDURAZYME showed improvement in FVC and in 6MWT compared to placebo-treated patients (see **Table 1**).

Table 1: Primary Efficacy Outcomes

		ALDURAZYME N = 22	Placebo N = 23
Forced Vital Capacity (percent of predicted normal)			
Baseline	Mean ± s.d.	48 ±15	54 ±16
Week 26	Mean ± s.d.	50 ±17	51 ±13
Change from baseline to week 26	Mean ± s.d.	1±7	-3±7
	Median	1	-1
Difference between groups	Mean	4	
	Median (95% CI)	2 (0.4, 7)	p=0.02*
6-Minute Walk Distance (meters)			
Baseline	Mean ± s.d.	319 ± 131	367 ±114
Week 26	Mean ± s.d.	339± 127	348 ±129
Change from baseline to week 26	Mean ± s.d.	20 ± 69	-18 ± 67
	Median	28	-11
Difference between groups	Mean	38	
	Median (95% CI)	39 (-2, 79)	p=0.07*

* By Wilcoxon Rank Sum Test

Evaluations of bioactivity were changes in liver size and urinary GAG levels. Liver size and urinary GAG levels decreased in patients treated with ALDURAZYME compared to patients treated with placebo. No subject in the group receiving ALDURAZYME reached the normal range for urinary GAG levels during this 6-month study.

All 45 patients received open-label ALDURAZYME for 36 weeks following the double-blind period. Maintenance of mean FVC and an additional increase in mean 6MWT distance were observed compared to the start of the open-label period among patients who were initially randomized to and then continued to receive ALDURAZYME. Among patients who had been initially randomized to placebo, improvements from baseline in mean FVC and 6MWT distance were observed compared to the start of the open-label period.

INDICATIONS AND USAGE

ALDURAZYME is indicated for patients with Hurler and Hurler-Scheie forms of Mucopolysaccharidosis I (MPS I) and for patients with the Scheie form who have moderate to severe symptoms. The risks and benefits of treating mildly affected patients with the Scheie form have not been established.

ALDURAZYME has been shown to improve pulmonary function and walking capacity. ALDURAZYME has not been evaluated for effects on the central nervous system manifestations of the disorder.

CONTRAINDICATIONS

There are no known contraindications to the use of ALDURAZYME.

WARNINGS

Hypersensitivity Reactions

Patients treated with ALDURAZYME may develop infusion-related hypersensitivity reactions (see **ADVERSE REACTIONS**). In the clinical studies, one patient developed an anaphylactic reaction approximately three hours after the initiation of the infusion. The reaction consisted of urticaria and airway obstruction. Resuscitation required an emergency tracheostomy. This patient's pre-existing MPS I related upper airway obstruction may have contributed to the severity of this reaction (see **ADVERSE REACTIONS: Infusion-Related Reactions** and **Immunogenicity**).

Some infusion-related reactions may be ameliorated by slowing the rate of infusion or treatment with additional antipyretics and/or antihistamines. If severe hypersensitivity or anaphylactic reactions occur, immediately discontinue the infusion of ALDURAZYME and initiate appropriate treatment. Caution should be exercised if epinephrine is being considered for use in patients with MPS I due to the increased prevalence of coronary artery disease in these patients.

The risks and benefits of re-administering ALDURAZYME following a severe hypersensitivity or anaphylactic reaction should be considered. Extreme care should be exercised, with appropriate resuscitation measures available, if the decision is made to re-administer the product.

PRECAUTIONS

General

Patients should receive antipyretics and/or antihistamines prior to infusion (see **WARNINGS** and **ADVERSE REACTIONS**). If an infusion reaction occurs, regardless of pre-treatment, decreasing the infusion rate, temporarily stopping the infusion, and/or administration of additional antipyretics and/or antihistamines may ameliorate the symptoms.

Information for Patients

Patients should be informed that a registry for MPS I patients has been established in order to better understand the variability and progression of MPS I disease, and to continue to monitor and evaluate treatments. Patients should be encouraged to participate and advised that their participation may involve long-term follow-up. Information regarding the registry program may be found at www.MPSIregistry.com or by calling (800) 745-4447.

Drug Interactions

No formal drug interaction studies have been conducted.

Carcinogenesis, Mutagenesis, Impairment of Fertility

Studies to assess the mutagenic and carcinogenic potential of ALDURAZYME have not been conducted.

Reproductive studies in rats have not demonstrated impairment of fertility (see **PRECAUTIONS: Pregnancy**).

Pregnancy: Category B

Reproduction studies have been performed in male and female rats at doses up to 6.2 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to ALDURAZYME. However, there are no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, ALDURAZYME should be used during pregnancy only if clearly needed.

Nursing Mothers

It is not known whether the drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when ALDURAZYME is administered to a nursing woman (see **PRECAUTIONS: Information for Patients** regarding a registry program. Nursing women are encouraged to participate in this program.).

Pediatric Use

Patients younger than 5 were not included in the clinical studies because of inability to comply with efficacy outcome assessments. It is not known if children younger than 5 respond differently from older children.

Geriatric Use

Clinical studies of ALDURAZYME did not include patients aged 65 and over. It is not known whether they respond differently from younger patients.

ADVERSE REACTIONS

The most serious adverse reaction reported with ALDURAZYME was an anaphylactic reaction consisting of urticaria and airway obstruction, which occurred in one patient. Pre-existing upper airway obstruction may have contributed to the severity of the reaction (see **WARNINGS: Hypersensitivity Reactions**).

The most common adverse reactions associated with ALDURAZYME treatment in the clinical studies were upper respiratory tract infection, rash, and injection site reaction.

The most common adverse reactions requiring intervention were infusion-related reactions, particularly flushing. Most infusion-related reactions requiring intervention were ameliorated with slowing of the infusion rate, temporarily stopping the infusion, and/or administering additional antipyretics and/or antihistamines.

The data described below reflect exposure to 0.58 mg/kg of ALDURAZYME for 26 weeks in a placebo-controlled double-blind study in 45 patients with MPS I (N=22 ALDURAZYME, and N=23 placebo). All 45 patients continued into an open-label study of ALDURAZYME treatment for an additional 36 weeks. An additional 10 patients participated in a Phase 1 open-label study with continued infusions for up to 3 years. The population in the placebo-controlled study was evenly distributed for gender (N=23 females and 22 males) and ranged in ages from 6 to 43 years. Of the 45 patients in the placebo-controlled study, 1 was clinically assessed as having Hurler form, 37 Hurler-Scheie, and 7 Scheie. All patients were treated with antipyretics and antihistamines prior to the infusions.

Because clinical trials are conducted under widely varying and controlled conditions, the observed adverse reaction rates may not predict the rates observed in patients in clinical practice.

Table 2 enumerates adverse events and selected laboratory abnormalities that occurred during the placebo-controlled trial in at least 2 patients more in the ALDURAZYME group than was observed in the placebo group. Reported adverse events have been classified using standard WHOART terms. Observed adverse events in the Phase 1 study and the open-label treatment period following the controlled study were not different in nature or severity.

Table 2: Number and (%) of Patients with Adverse Events and Selected Laboratory Abnormalities in the Placebo-Controlled Study

Adverse Event	Placebo (N = 23)	ALDURAZYME (N = 22)
Respiratory System		
Upper respiratory tract infection	4 (17)	7 (32)
Body as a Whole		
Chest pain	0	2 (9)
Nervous System		
Hyperreflexia	0	3 (14)
Paresthesia	1 (4)	3 (14)
Skin and Appendages		
Rash	5 (22)	8 (36)
Resistance Mechanism		
Abscess	0	2 (9)
Liver and Biliary System		
Bilirubinemia	0	2 (9)
Vascular		
Vein disorder	1 (4)	3 (14)
Urinary System		
Facial edema	0	2 (9)
Cardiovascular, General		
Hypotension	0	2 (9)
Dependent edema	0	2 (9)
Vision		
Corneal opacity	0	2 (9)
Application Site		
Injection site pain	0	2 (9)
Injection site reaction	2 (9)	4 (18)
Platelet, Bleeding and Clotting		
Thrombocytopenia	0	2 (9)

Infusion-Related Reactions

Infusion-related reactions were reported in 7 of 22 patients treated with ALDURAZYME. Infusion-related reactions were not significantly different between the ALDURAZYME treatment group and the placebo group who received infusions of diluent and all components of ALDURAZYME except the laronidase enzyme. The most common infusion-related reactions included flushing, fever, headache and rash. Flushing occurred in 5 patients (23%) receiving ALDURAZYME; the other reactions were less frequent. All reactions were mild to moderate in severity. The frequency of infusion-related reactions decreased with continued use during the open-label extended use period. There was one case of anaphylaxis during the open-label extension period (see **WARNINGS** and **ADVERSE REACTIONS: Immunogenicity**). Less common infusion-related reactions include cough, bronchospasm, dyspnea, urticaria, angioedema and pruritus.

Immunogenicity

Fifty of 55 patients (91%) treated with ALDURAZYME were positive for antibodies to laronidase. The clinical significance of antibodies to ALDURAZYME is not known, including the potential for product neutralization.

The data reflect the percentage of patients whose test results were considered positive for antibodies to ALDURAZYME using an enzyme-linked immunosorbent assay (ELISA) for laronidase-specific IgG binding antibodies, and are highly dependent on the sensitivity and specificity of the assay. Additionally, the observed incidence of antibodies in an assay may be influenced by several factors including sample handling, timing of sample collection, concomitant medications, and underlying disease. For these reasons, comparison of the incidence of antibodies to ALDURAZYME with the incidence of antibodies to other products may be misleading.

Four patients in the controlled study who experienced severe infusion-related reactions were tested for ALDURAZYME specific IgE antibodies and complement activation. IgE testing was performed by ELISA and complement activation was measured by the Quidel Enzyme Immunoassay. One of the four patients had an anaphylactic reaction consisting of urticaria and airway obstruction and tested positive for both ALDURAZYME specific IgE binding antibodies and complement activation (see **WARNINGS: Hypersensitivity Reactions**).

Other hypersensitivity reactions were also seen in patients receiving ALDURAZYME (see **ADVERSE REACTIONS: Infusion-Related Reactions**).

OVERDOSAGE

There is no experience with overdoses of ALDURAZYME.

DOSAGE AND ADMINISTRATION

The recommended dosage regimen of ALDURAZYME is 0.58 mg/kg of body weight administered once-weekly as an intravenous infusion.

Pretreatment with antipyretics and/or antihistamines is recommended 60 minutes prior to the start of the infusion (see **PRECAUTIONS: General**).

The total volume of the infusion is determined by the patient's body weight and should be delivered over approximately 3 to 4 hours. Patients with a body weight of 20 kg or less should receive a total volume of 100 mL. Patients with a body weight of greater than 20 kg should receive a total volume of 250 mL. The initial infusion rate of 10 mcg/kg/hr may be incrementally increased every 15 minutes during the first hour, as tolerated, until a maximum infusion rate of 200 mcg/kg/hr is reached. The maximum rate is then maintained for the remainder of the infusion (2-3 hours).

For Patients Weighing 20 kg or Less

Total Volume of ALDURAZYME Infusion = 100 mL	
2 mL/hr x 15 minutes (10 mcg/kg/hr)	Obtain vital signs, if stable then increase the rate to...
4 mL/hr x 15 minutes (20 mcg/kg/hr)	Obtain vital signs, if stable then increase the rate to...
8 mL/hr x 15 minutes (50 mcg/kg/hr)	Obtain vital signs, if stable then increase the rate to...
16 mL/hr x 15 minutes (100 mcg/kg/hr)	Obtain vital signs, if stable then increase the rate to...
32 mL/hr x ~3 hours (200 mcg/kg/hr)	For the remainder of the infusion.

For Patients Weighing Greater than 20 kg

Total Volume of ALDURAZYME Infusion = 250 mL	
5 mL/hr x 15 minutes (10 mcg/kg/hr)	Obtain vital signs, if stable then increase the rate to...
10 mL/hr x 15 minutes (20 mcg/kg/hr)	Obtain vital signs, if stable then increase the rate to...
20 mL/hr x 15 minutes (50 mcg/kg/hr)	Obtain vital signs, if stable then increase the rate to...
40 mL/hr x 15 minutes (100 mcg/kg/hr)	Obtain vital signs, if stable then increase the rate to...
80 mL/hr x ~3 hours (200 mcg/kg/hr)	For the remainder of the infusion.

Each vial of ALDURAZYME provides 2.9 mg of laronidase in 5.0 mL of solution and is intended for single use only. Do not use the vial more than one time. The concentrated solution for infusion must be diluted with 0.1% Albumin (Human) in 0.9% Sodium Chloride Injection, USP using aseptic techniques. ALDURAZYME should be prepared using PVC Containers and administered with a PVC infusion set equipped with an in-line, low protein binding 0.2 micrometer (μm) filter. There is no information on the compatibility of diluted ALDURAZYME with glass containers.

Instructions for Use (Aseptic Techniques)

1. Determine the number of vials to be diluted based on the individual patient's weight and the recommended dose of 0.58 mg/kg [Patient's weight (kg) x 1 mL/kg of ALDURAZYME = Total # mL of ALDURAZYME, then Total # of mL of ALDURAZYME ÷ 5 mL per Vial = Total # of Vials]. Round up to the nearest whole vial. Remove the required number of vials from the refrigerator to allow them to reach room temperature. Do not heat or microwave vials.
2. Before withdrawing the ALDURAZYME from the vial, visually inspect each vial for particulate matter and discoloration. The ALDURAZYME solution should be clear to slightly opalescent and colorless to pale yellow. A few translucent particles may be present. Do not use if the solution is discolored or if there is particulate matter in the solution.
3. Determine the total volume of the infusion to be used based on the patient's body weight. The total final volume should be either 100 mL (if weight is less than or equal to 20 kg) or 250 mL (if weight is greater than 20 kg).
4. Using the chart below, prepare an infusion bag of 0.1% Albumin (Human) in 0.9% Sodium Chloride Injection, USP. Remove and discard a volume of 0.9% Sodium Chloride Injection, USP equal to the volume of Albumin (Human) to be added to the infusion bag. Add the appropriate volume of Albumin (Human) to the infusion bag and gently rotate the infusion bag to ensure proper distribution of the Albumin.

Total Volume of ALDURAZYME Infusion	Volume of Albumin (Human) 5% to be Added	Volume of Albumin (Human) 25% to be Added
100 mL	2 mL	0.4 mL
250 mL	5 mL	1 mL

5. Withdraw and discard a volume of the 0.1% Albumin (Human) in 0.9% Sodium Chloride Injection, USP from the infusion bag, equal to the volume of ALDURAZYME concentrate to be added.

6. Slowly withdraw the calculated volume of ALDURAZYME from the appropriate number of vials using caution to avoid excessive agitation. Do not use a filter needle, as this may cause agitation. Agitation may denature ALDURAZYME, rendering it biologically inactive.
7. Slowly add the ALDURAZYME solution to the 0.1% Albumin (Human) in 0.9% Sodium Chloride Injection, USP using care to avoid agitation of the solutions. Do not use a filter needle.
8. Gently rotate the infusion bag to ensure proper distribution of ALDURAZYME. Do not shake the solution.

ALDURAZYME does not contain any preservatives, therefore after dilution with saline in the infusion bags, any unused product or waste material should be discarded and disposed of in accordance with local requirements.

ALDURAZYME must not be mixed with other medicinal products in the same infusion.

The compatibility of ALDURAZYME in solution with other products has not been evaluated.

STORAGE

Store ALDURAZYME under refrigeration at 2°C to 8°C (36°F to 46°F). DO NOT FREEZE OR SHAKE. DO NOT USE ALDURAZYME after the expiration date on the vial. This product contains no preservatives.

The diluted solution should be used immediately. If immediate use is not possible, the diluted solution should be stored refrigerated at 2°C to 8°C (36°F to 46°F). The in-use storage should not be longer than 36 hours from the time of preparation to completion of administration. Room temperature storage of diluted solution is not recommended.

HOW SUPPLIED

ALDURAZYME is supplied as a sterile solution in clear Type I glass 5 mL vials (2.9 mg laronidase per 5 mL). The closure consists of a siliconized butyl stopper and an aluminum seal with a plastic flip-off cap.

NDC 58468-0070-1

Rx Only

ALDURAZYME is manufactured by:
BioMarin Pharmaceutical Inc.
371 Bel Marin Keys Blvd.
Suite 210
Novato, CA 94949
US License Number 1649

ALDURAZYME is distributed by:
Genzyme Corporation
One Kendall Square
Cambridge, MA 02139
1-800-745-4447 (phone)

STN125058/0

TOXICOLOGIST'S REVIEW

BLA: STN 125058
SPONSOR: Biomarin Pharmaceutical Inc.
PRODUCT: recombinant human a-iduronidase; rhIDU; laronidase; Aldurazyme™
FORMULATION/CHEMISTRY: Isolated from cell culture supernatant after growth of CHO cells transfected with a recombinant expression vector encoded for rhIDU. Formulated as a sterile, liquid solution of polysorbate 80 (10 µg/ml, 0.05 mg) in a sodium chloride (150 mM, 43.9 mg) and sodium phosphate buffer (92 mM, 63.5 mg). The drug product is to be diluted for intravenous administration with between 100ml to 250ml of 0.9% sodium chloride solution. The final product (2.90 mg/ vial, 100 units/ml), is reconstituted with 5 mL of Water for Injection USP.
PROPOSED INDICATION: Long term enzyme replacement therapy in patients with Mucopolysaccharidosis I (MPS I; a-l-iduronidase deficiency) to treat the non-central nervous system manifestations of the disease
ABBREVIATIONS: recombinant human a-iduronidase = rhIDU, intravenous = IV, glycosaminoglycan = GAG; MPS I = Mucopolysaccharidosis I, (α-L-iduronidase deficiency)

Application History

BL125058/0.00: Original submission of rolling BLA 26-JUL-2002
BL125058/0.01: Preclinical Toxicology Update 04-SEP-2002
BL125058/0.02: Neutralizing Antibody Data for Phase 3 (ALID-003-99) 10-OCT -2002
BL125058/0.03: Additional Dataset % Predicted Normal FVC Phase 3 (ALID-003-99) 17-OCT -2002
BL125058/0.04: 36-Week Efficacy Data for Phase 3 Ext. (ALID-006-01) 24-OCT-2002
BL125058/0.05: 120-Day Update 05-DEC-2002
BL125058/0.06: Responses to the Discipline Review Letter for the CMC 09-DEC -2002
BL125058/0.07: 120-Day Update: CRTs & Programs 12-DEC-2002
BL125058/0.08: Clarification: Number of Bioreactors for Cell Culture of Laronidase 19-DEC –2002

CROSS-REFERENCES: BB-IND ----------

Table of Contents

Introduction ..... *p. 02*
Preclinical Pharmacology ..... *p. 05*
PK/ADME Studies ..... *p. 08*
Acute Toxicity Studies ..... *p. 11*
Multidose Toxicity Studies ..... *p. 11*
Reproductive/Developmental Toxicity Studies ..... *p. 13*
Mutagenicity Studies ..... *p. 14*
Carcinogenicity Studies ..... *p. 15*
Safety Pharmacology Studies ..... *p. 15*
Conclusion ..... *p. 15*

Introduction

The target agent, Aldurazyme™ (laronidase) is a recombinant form of human a-iduronidase enzyme, which is a monomeric protein with a molecular weight of 83kD by ---------- analysis. Laronidase is a 628 amino acid lysosomal hydrolase that cleaves iduronic acid residues from the non-reducing ends of heparan sulfate and dermatan sulfate. The enzyme is a single polypeptide chain of molecular mass ---------- ---------- and a molecular formula of ---------- contains six asparagine-linked glycosylation sites, two of which carry the bis mannose-6-phosphate oligomannose$_7$ oligosaccharide that binds the target cell surface receptor. The apparent increase in molecular mass of -- kD over that predicted by the amino acid sequence is a result of posttranslational modifications.

Mucopolysaccharidosis I (MPS I) is an autosomal recessive disorder located at 4p16.3. In the human, α-L-iduronidase deficiency results in a spectrum of clinical manifestations and disease severities. These manifestations are directly related to accumulation of glycosaminoglycans (GAGs), primarily dermatan and heparan sulfate, as a result of the lysosomal enzyme deficiency. The clinical spectrum is conventionally categorized into three overlapping clinical syndromes that vary in clinical severity. These are in order of decreasing clinical severity, Hurler syndrome (MPS1H), Hurler-Scheie Syndrome (MPS IH-S), and Scheie Syndrome (MPS-IS). The three syndromes are indistinguishable on the basis of the routine clinical chemistry measures as all three exhibit only minimal enzyme activity and all show elevated urinary GAG levels the ranges of which overlap. Further although the classical diagnostic criteria are clinical,

they also overlap and the designation of an individual into a particular diagnostic category is somewhat subjective. In general Hurler patients present within the first year multiple of the following findings: coarse facies, skeletal deformities, prominent forehead, hernia (umbilical or inguinal), enlarged tongue, short stature, joint stiffness, acute cardiomyopathy associated with endocardial fibroelastosis, developmental delay that progressively increases, deafness, recurring upper respiratory tract and ear infections, obstructive airway disease, sleep apnea, noisy breathing, persistent copious nasal discharge, corneal clouding, and occasionally communicating hydrocephalus associated with increased intracranial pressure. Death usually occurs within the first decade of life. Patients classified as Hurler-Sheie patients typically present later (between the ages of 3 and 8) with milder symptoms. These symptoms include hepatosplenomegaly, obstructive airway disease and sleep apnea, recurring respiratory infections, dysostosis multiplex, short stature, characteristic coarse facies, corneal clouding, joint stiffness, deafness, and valvular heart disease. The life expectancy for these patients is to reach young adulthood. Unlike Hurler patients, Hurler-Scheie patients achieve normal developmental milestones. The patients with the mildest MPS I deficiency phenotype, Sheie Syndrome are usually diagnosed in the teen years and the presenting symptoms are often joint stiffness, aortic valve disease, mild hepatosplenomegaly or corneal clouding. These patients have little or no neurological manifestations, a normal stature and may live a normal lifespan with only minimal clinical symptoms and few restrictions on activities of daily living.

The proposed clinical indication for Aldurazyme™ (laronidase) is "long term enzyme replacement therapy in patients with Mucopolysaccharidosis I (MPS I; α-L-iduronidase deficiency) to treat the non-central nervous system manifestations of the disease". In the submitted package insert the sponsor recommended the following dosage regimen-

"The recommended dosage regimen of Aldurazyme™ is 100 U/kg (0.58 mg/kg) of actual body weight administered once weekly as an intravenous infusion."

With initial administrations of Aldurazyme™, it is recommended that patients be administered pretreatment medications approximately 60 minutes prior to the start of the infusion. If clinically indicated, the administration of pretreatment medications should continue with subsequent infusions of Aldurazyme™.

The total volume of the infusion is determined by the patient's actual body weight and should be delivered over approximately 3 to 4 hours. Patients with an actual body weight of 20 kg or less should receive a total volume of 100 mL. Patients with an actual body weight of greater than 20 kg should receive a total volume of 250 mL. The initial infusion rate of 2 U/kg/hr may be incrementally increased every 15 minutes during the first hour, as tolerated, until a maximum infusion rate of 43 U/kg/hr is reached. The maximum rate is then maintained for the remainder of the infusion (2-3 hours).

Each vial of Aldurazyme™ contains 500 U (100 U/mL; 0.58 mg/mL) of laronidase and is intended for single use only. The concentrate for solution for infusion must be diluted with 0.1% Human Serum Albumin in 0.9% Sodium Chloride for Injection, USP using aseptic techniques. In the absence of stability studies using glass containers, it is recommended that Aldurazyme™ be prepared and administered using PVC Containers. It is recommended that the Aldurazyme™ solution be administered with a PVC infusion set equipped with an in-line, low protein binding 0.2 micrometer (μm) filter.

The primary trial used to demonstrate Aldurazyme™ was a single randomized, placebo-controlled clinical trial of 45 MPS I patients, of whom 1 was classified as having the Hurler form, 37 Hurler-Scheie, and 7 Scheie. All patients had a baseline forced vital capacity (FVC) less than or equal to 80% of predicted. Patients received Aldurazyme™ at 0.58mg/kg or placebo once weekly for 26 weeks. In clinical studies the most significant adverse reactions were infusion related-reactions of varying clinical intensities as were seen in the preclinical studies. As a result in the Phase 3 Studies, all patients were pretreated prior to each infusion with age-appropriate dosages of antihistamines and antipyretics, such as diphenhydramine or hydroxyzine and acetaminophen or ibuprofen, respectively.

The principal efficacy outcome assessments were FVC and distance walked in 6 minutes (6 minute walk test, 6MWT). After 26 weeks, AldurazymeTM-treated patients showed improvement in FVC and in the 6MWT compared to placebo-treated patients. Evaluations of bioactivity were changes in liver size and urinary GAG levels. Liver size and urinary GAG levels were decreased in Aldurazyme™ treated patients compared to placebo-treated patients. No subject in the Aldurazyme™-treated group reached the normal range for urinary GAG levels during this study.

Preclinical Pharmacology Studies

Notes of clarification

Note that during preclinical development of rhIDU drug product, the assay for a-L-iduronidase activity was changed to make it more reproducible and robust and the definition of a unit was changed to be more conventional. In addition, the —

—The sponsor provided an overview of the doses administered in all studies with dose conversions for all studies in the BLA submission. For clarity, doses are presented in mg/kg utilizing the new units throughout this BLA review, in order to facilitate comparison to the recommended human dose.

Also note that the dates presented with each study are the dates the report was issued, not the date of study completion when report issued date is available. This convention will be used throughout this BLA review.

Assay for rhIDU activity in animal studies

List of Pharmacology Studies

1. IDU-PC-002: Short-term Intravenous Infusion Study of Recombinant Human α-L-Iduronidase in a Single Dog, non-GLP, Conducted at----------, 11/93, Lots no. ----------

2. IDU-PC-003: Acute intravenous infusion study of recombinant human α-L-Iduronidase in a single dog; non-GLP, Conducted at----------, 4/95, Lots no. -----

3. IDU-PC-004: Subchronic Intravenous Infusion Study of Recombinant Human α-L-Iduronidase in Dogs: 4/94, non-GLP, Conducted at----------, Lots nos. -----

4. IDU-PC-005: Thirteen-Month Intravenous Infusion Study of Recombinant Human a-L-Iduronidase in a Dog: non-GLP, Conducted at----------, 1/95, Lot nos. -----

5. IDU-PC-006: 74-Week Intravenous Infusion Study of Recombinant Human a-L-Iduronidase in Dogs: 3/99, non-GLP, Conducted at----------, Lot nos. ----------

6. IDU-PC-008: Comparison of Continuous and Bolus Intravenous Infusions of Recombinant Human α-L-Iduronidase in Dogs, non-GLP, Conducted at ---------- ----------, 02/01, Lot no. ---.

7. Kakkis et al.: Enzyme replacement therapy in feline mucopolysaccharidosis I, N/A, Lot nos. -----

Review of Pharmacology Studies

1. IDU-PC-002: Short-term Intravenous Infusion Study of Recombinant Human α-L-Iduronidase in a Single Dog: Multi-step intravenous dose regimen study in a single dog. Part 1- 0.116mg/kg IV every other day for 7 doses (12 days), part 2 - IV three doses every other day for 5 days starting two months after the first dose, part 3- one IV dose (0.116 mg/kg) five months after the first dose. Levels of IDU increased in liver biopsy after treatment. Histopathology reportedly shows decreased cellular vacuolation in live and normalization of the histology of hepatocytes and Kupffer cells.

2. IDU-PC-003 One control female MPS I dog and two laronidase treated 0.58 mg/kg IV (one male and one female) MPSI dogs every other day for 5 doses (days1, 2,5,8,10) with sacrifice on day 12. Levels of IDU increased relative to untreated MPS I dogs in all tissues sampled (liver, spleen, lung, kidney, cerebrum, heart valve, myocardium, lymph node, and cornea).

3. IDU-PC-004: Subchronic Intravenous Infusion Study of Recombinant Human α-L- Iduronidase in Dogs. 2 untreated MPS I dogs and 3 MPS I dogs treated intravenously with 0.116 mg/kg rhIDU weekly for 3 months. Levels of IDU in liver greater than that in normal dog liver and spleen, other tissues less than normal dog but elevated relative to MPS I control. Histopathologic analysis showed decreased cellular vacuolation in liver, kidney and spleen.

4. IDU-PC-005: Thirteen-Month Intravenous Infusion Study of Recombinant Human α-L-Iduronidase in a Dog. Study of weekly intravenous infusion of 0.116 mg/kg for 74 weeks. Activity of a-L-Iduronidase increased in all tissues measured versus control levels in MPS I dog. Levels of IDU in liver greater than that in normal dog liver, other tissues less than normal dog but elevated relative to MPS I control. The GAG accumulation was decreased but still above normal. Histopathologic analysis showed persistent GAG accumulation in liver, kidney, adrenal gland, lung, lymph node, small intestine, spleen, synovium, gall bladder despite decreased cellular vacuolation.

5. IDU-PC-006: 74-Week Intravenous Infusion Study of Recombinant Human α-L-Iduronidase in Dogs: Uncontrolled intravenous dose-ranging study in MPSI dog model. Two dogs dosed with 0.058-0.58 mg/kg IV 1-2 times a week for weeks 1-7, then with 0.58 mg/kg IV 3 times a week for weeks 8-46, then once per week with 0.58 mg/kg IV for weeks 47-74. Results show a-L-Iduronidase activity approaching normal in liver, intestine, kidney, lung, lymph nodes, spleen, myocardium, synovium, and rib cartilage, all other tissues are less than normal. GAG accumulation decreased in all tissues relative to beginning and still above normal. Histopathology show depleted accumulation in macrophages in all tissues except CNS and dense connective tissue. Clinical improvement noted.

6. IDU-PC-008: Comparison of Continuous and Bolus Intravenous Infusions of Recombinant Human α-L-Iduronidase in Dogs: Two untreated and seven treated MPS I dogs were treated intravenously with laronidase. Two animals treated with 0.58 mg/kg and three with 2.32 mg/kg for continuous infusion for 10-39 weeks, and two dogs treated weekly for 10 weeks. Results show a-l-Iduronidase activity increased in all tissues measured relative to MPSI control animals. Levels were greater than normal canine levels in liver, spleen, kidney, lymph nodes, rib cartilage, synovium, and tracheal cartilage after both continuous and bolus infusions. GAG accumulation decreased to within or > 2-fold the normal range for kidney, liver, lung, lymph nodes, spleen, and synovium after more than one dosing interval. No reductions seen in heart valve or brain. Histopathologic information in macrophages, lymph nodes, spleen and liver reduced more by bolus than continuous infusion and with a positive dose response. No decrease in GAG vacuolation in CNS or dense connective tissue.

7. Kakkis et al., Mol. Genet. Metab. 2001. Dose ranging study in MPS I deficient cats. MPS I cats were treated intravenously for up to six months. Three were treated with 0.116 mg/kg weekly for three months, one was treated with 0.58 mg/kg IV weekly for three months in the first part of the study. In the second part of the study, one cat was treated with 0.116 mg/kg IV weekly and one cat was treated with 0.58 mg/kg IV weekly for six months. The a-L-Iduronidase activity was increased relative to controls with all tissues measured except brain, and rib cartilage, GAG accumulation was decreased to the normal range in liver, spleen and lung.

PK/ADME Studies

List of PK/ADME Studies

1. IDU-PC-001: Clearance and Tissue Distribution Study of Recombinant Human α-L-Iduronidase in Dogs: non- GLP, Conducted by ----------9/93, Lots no. ----------

2. IDU-PC-008: Comparison of Continuous and Bolus Intravenous Infusions of Recombinant Human a-L-Iduronidase in Dogs, non-GLP, Conducted at ---------- ----------, 2/01, Lot no. ----.

Review of PK/ADME Studies

1. IDU-PC-001: Clearance and Tissue Distribution Study of Recombinant Human α-L-Iduronidase in Dogs: One female MPS I dog was treated daily with 0.116 mg/kg for days 1 and 2. Result: Biphasic clearance: $t_{1/2}$ α = 0.9 minutes, $t_{1/2}$ β = 18.9 minutes.

2. IDU-PC-008: Comparison of Continuous and Bolus Intravenous Infusions of Recombinant Human a-L-Iduronidase in Dogs: Two MPS I dogs (one male, one female) were treated once during weeks 2 and 10 with 2.32 mg/kg IV. Results: Week 2- Biphasic clearance, $t_{1/2}$ α = 0.9 minutes, $t_{1/2}$ β = 59.5 and 94.9 minutes. Week 10- Monophasic clearance, $t_{1/2}$ = 66.2 and 23.8 minutes. V_c = approximately 60 ml/kg. AUC (U/ml-hr) increased from week 2 (269 and 407) to week (13364 and 2559). Clearance from (ml/kg/min) decreased from week 2 (31.0 and 20.5) to week 10 (0.62 and 3.26).

Preclinical Toxicology Studies

List of Preclinical Toxicology Studies:

1. IDU-PC-007: An acute intravenous toxicity study in rats. GLP, conducted at------ ---------- No. 0406RB31.001, 3/01, Lot No.----------.

2. IDU-PC-009: 26-week intravenous infusion toxicity study with recombinant human a-L-Iduronidase in monkeys with a 2-week recovery: ----------monkeys- ----------, GLP, Conducted at ----------. 6354-122), 8/02, Lot No. PD-01-01 (from Lot ----------)

3. IDU-PC-011: Effect of repeat intravenous administration of recombinant human α-L-Iduronidase with and without canine serum albumin to ------ dogs (---------- No. 6354-130), GLP, Conducted at ----------, 1/02, Lot No. PD-01-01 (from Lot ----------.

4. IDU-PC-012: A one-day evaluation of the hemodynamic effects of the administration of Aldurazyme™ (laronidase for injection) to dogs during a 4-hour infusion. GLP, conducted by ----------No. R-032), 9/00, Lot nos. ----------

5. IDU-PC-013: Intravenous fertility and general reproduction toxicity study of alpha-L-Iduronidase in rats: GLP, Conducted by ---------- Protocol Number 907-008, Biomarin Study Number 01035, 8/02, Lots no. ---------- ----------

6. IDU-PC-014: Intravenous developmental toxicity study of alpha-L-Iduronidase in rats; GLP, Conducted by ----------No. 907-007; Biomarin Study Number: 01037, 8/02, Lots no. ----------

Review of Preclinical Toxicology Studies:

Acute Toxicity Studies

1. **IDU-PC-007: An acute intravenous toxicity study in rats:**
   Species: ----------rats
   Dose Levels: 0, 0.29, 0.58, 5.8 mg/kg
   Route Duration: single IV bolus, + kills on day 15.
   Methods: Clinical signs, BW, clinical pathology, gross & histopathology.
   Findings: No abnormalities in BWs, clinical pathology, organ weights. Incidental gross finding of single pale focus in liver in a male (5.8mg/kg group). Increase in number (but not incidence of sporadic findings of small foci of hepatocellular necrosis).

   The NOAEL was $\leq$ 0.58 mg/kg

2. **IDU-PC-012: A one-day evaluation of the hemodynamic effects of the administration of Aldurazyme™ (laronidase for injection) to dogs during a 4-hour infusion: General GLP adherence**
   Species: ---------- dogs (2 females)
   Dose Levels: 0.7 mg/kg, 3.9 mg/kg, 4.9 mg/kg
   Route Duration: IV x 2 approximately 4 hour infusion. Low and high doses in each animal after a 30 minute space (one high dose 3.9 mg/kg & one high dose 4.9 mg/kg).
   Methods: Cardiovascular (EKG, BP) monitoring, kill same day as treatment 30 minutes after high dose treatment.
   Findings: No treatment related abnormalities in EKG or HR.

   The NOAEL was $\leq$ 4.9 mg/kg

Multidose Toxicity Studies

1. **IDU-PC-009: 26-week intravenous infusion toxicity study with recombinant human a-L-Iduronidase in monkeys with a 2-week recovery:** ---------- **monkeys----------:**

Species: ---------- monkeys ----------
Dose Levels: 0, 0.166, 1.659, 16.588 (5/sex/group)
Route Duration: IV bolus (7 hour infusion) weekly for 26 weeks.
Methods: Clinical signs, BW, clinical pathology, gross & histopathology, ophthalmic examinations, antibody analysis, toxicokinetics.
Findings: Monkey (152259) developed hypersensitivity with edema around eyes and muzzle half way through the fourth dose. No treatment-related abnormalities in food consumptions, organ weights, sperm counts or morphology, gross, or histopathology. Decrease in BW of females in low and mid-dose (0.166, 1.659 mg/kg) at week 26, no effect in high dose group.
Clinical pathology- All monkeys in the high dose groups (male & female) 16.588 mg/kg have increased total leukocyte, lymphocytes and eosinophil counts, and monocyte counts (female) at one or more treatment groups. The changes (except eosinophil in male, and monocyte in female) resolved during the recovery period. No specific clinical sequelae of these changes were seen.
Antibody assays-
Approximately half the animals had increased levels of antibodies at 26 weeks versus the levels at 13 weeks. The animals that developed antibodies to rhIDU developed them to various levels (61.2-8843.9 A_{450} units per µL serum at week 26). The response does not appear to be dose related. There was no difference in antibody levels due to sex.
Pharmacokinetics- Serum rhIDU levels were insufficient at 0.166 mg/kg to perform pK analysis. Decreased AUC in 1.659 mg/kg group at 1, 13 and 26 weeks, but not seen at high dose (16.588 mg/kg). Liver levels of rhIDU determined and found to be dose-related.

The NOAEL was 1.659 mg/kg

2. **IDU-PC-011: Effect of repeat intravenous administration of recombinant human α-L-Iduronidase with and without canine serum albumin to —— dogs:**
Species: ------ dog
Dose Levels: 0, 1.6 mg/kg
Route Duration: IV 4 hour infusion once a week for 8 weeks.
Methods: Clinical signs, BW.
Findings: All formulations induced facial edema, emesis, mucoid stools and/or

excessive salivation starting at the third dose. The severity of clinical effects were ranked thus: rhIDU> rhIDU with dog serum> rhIDU with Tween 80.

Reproductive/Developmental Toxicity Studies

1. **IDU-PC-013: Intravenous fertility and general reproduction toxicity study of alpha-L-Iduronidase in rats:**

   Species: ----------rats (25/sex/group)

   Dose Levels: 0, 0.036, 0.36, 3.6 mg/kg/day for rhIDU and 5mg/kg for diphenhydramine (DPH) pretreatment for all rhIDU doses including a vehicle control.

   Route Duration: IV bolus for rhIDU and DPH. Male rats – rhIDU daily for 21 days prior to cohabitation for a total of 28 days (DPH days 9-35). Female rats – Daily rhIDU 15 days before cohabitation and until DG7 (DPH day 1 of study until DG7). Male sac at day after mating, female at DG 21.

   Methods: Clinical signs, BW, food consumption, vaginal smears, sperm evaluation, gross & histopathology.

   Findings: Female: Reduction of increase in weight in DPH, 0.036, 3.6 mg/kg groups for DS 8 to 15. No Rx-related biological effects on clin signs, food consumption, gross path, estrous cycles – normal. Male: Testes/prostate/seminal vesicles/epididymides wts - comparable between groups. No adverse effects on sperm motility, sperm counts, sperm morphology

   Mating indices= 100%/100%/100%/100%/100% at 0/DPH (5)/0.036/0.36/3.60 mg/kg

   Pregnancy rates = 96%/87.5%/92%/91.7%/88% at 0/DPH (5)/0.036/0.36/3.60 mg/kg

   Mean percent preimplantation loss per rat [corpora lutea minus implants] = 12/10/12/17/10 at 0/DPH (5)/0.036/0.36/3.60 mg/kg

   Mean percent postimplantation loss per rat [implants minus live fetuses] = 2.5/3.6/3.9/3.2/4.2 at 0/DPH (5)/0.036/0.36/3.60 mg/kg

   Mean number of live fetuses per rat = 15.4/14.7/15/14/14.8 at 0/DPH(5)/0.036/0.36/3.60 mg/kg

2.**IDU-PC-014: Intravenous developmental toxicity study of alpha-L-Iduronidase in rats:**

Species: ---------- rats (25 female rats/group)

Dose Levels: 0, DPH (5), 0.036, 0.36, 3.6 mg/kg

Route Duration: Slow IV bolus daily on DGs 7 through 17.

Methods: Clinical signs, BWs, food consumption, vaginal smears, TK profile, pregnancy rate, uterine contents, ovary evaluation, gross evaluation, fetal exams

Findings: There were statistically significant reductions in body weight gains (DGs10-12) in the 0.36 and3.6 mg/kg/day groups and in the food consumption on days 15 to 18 in the 0.36 and 3.6 mg/kg groups. No adverse effects on clinical signs, estrous cycles, gross pathology

Mating indices= 100% - all groups

Pregnancy rates = 88%/96%/84%/96%/100% at 0/DPH (5)/0.036/0.36/3.6 mg/kg

Mean percent preimplantation loss per rat = 6.4%/6.3%/11%/8.3%/3.2% at 0/ DPH (5)/0.036/0.36/3.6 mg/kg

Mean percent postimplantation loss per rat = 3.2%/3.8%/2.9%/1.7%/2.4% at 0/ DPH (5)/0.036/0.36/3.6 mg/kg

Mean number of live fetuses per rat = 14.3/14/13.1/14.5/14.4 at 0/ DPH (5)/0.036/0.36/3.6 mg/kg

F1 Fetuses - No Rx-related effects on BWs, external, visceral, or skeletal anomalies

The NOEL was 0.036 mg/kg/day for parental toxicity and for fertility & reproductive performance. The NOEL for embryotoxicity was >3.6 mg/kg/day.

Mutagenicity Studies

No studies were performed.

Carcinogenicity Studies

No studies were performed.

Safety Pharmacology Studies

No studies were performed.

Conclusion

Aldurazyme™ is a highly purified and well-characterized 83 kD glycoprotein. It is isolated from cell culture supernatant after growth of CHO cells transfected with a recombinant expression vector encoded for rhIDU The proposed clinical indication is the long term enzyme replacement therapy in patients with Mucopolysaccharidosis I (MPS I; a-l-iduronidase deficiency) to treat the non-central nervous system manifestations of the disease. The safety, efficacy, pharmacokinetics and biodistribution of recombinant human a-L-iduronidase (rhIDU) have been evaluated using studies in four species (dog [MPS I and wild-type], MPS I cat, ---------- monkey, and ---- rat) of animals. The results of those studies demonstrate that rhIDU has an acceptable safety profile, with no consistent, treatment-related toxicity other than the immune reaction of the animals to a foreign protein and/or other constituent of the product. The most significant treatment-related finding seen in the preclinical studies was an anaphylactoid reaction that occurred in several dogs in early pharmacology studies. Reoccurrence of this event was prevented in most of the later studies by pharmacological pretreatment of the animals and changes in the dosing solution and regimen, however a significant infusion reaction occurred in the---------- monkey study during administration of the fourth dose. The animal had not been pretreated.

Two species (dog and cat) provide natural animal models of the MPS I disease, and a ---------- mouse model has been described (but not submitted in the BLA application)(Haskins 1997, He 1999, Clarke 1997). The naturally occurring feline MPS I model the disease is a result of a----------. The canine model is maintained in a mixed breed colony manifestations seen in naturally occurring disease is remarkably

similar in three species known to have natural disease, human, cat, and dog. The manifestations emanate from a marked deficiency of the lysosomal enzyme, α-L-iduronidase which is manifested universally by excessive urinary dermatan sulfate and heparan sulfate. The clinical features in all three species include facial dysmorphia, corneal clouding, cardiac valvular insufficiencies and bone disease. Felines affected by the disease do not manifest the growth delay that is a prominent feature in human and canine disease, but do survive to reproductive age, as do canines and humans. The sponsor has submitted seven studies in naturally occurring models of disease (six MPS I canine studies, one MPS I feline study) as pharmacodynamic studies to support the rationale of biological effectiveness in of the product, Aldurazyme™.

The MPS I canine studies submitted (IDU-PC-002, IDU-PC-003, IDU-PC-004, IDU-PC-005, IDU-PC-006, IDU-PC-008) are intravenous studies that explore dose and dosing regimens. The studies used a total of 28 dogs including controls. The consistent pharmacologic finding in these studies was that the treatment decreased GAG accumulations in the liver. This finding was seen across the 20U/kg/week to 100U/kg/week dose range and across various dosing regimens (single IV dosing, every other day dosing, and continuous infusions). Although longer duration studies (IDU-PC-005, IDU-PC-006) also reduce GAG accumulations in additional tissues such as kidney, spleen, adrenal gland, lymph nodes, small intestine, joint synovium, and gall bladder as measured by histology, histological evaluation failed to demonstrate a reduction in GAG accumulation in the CNS or in cartilage as measured by a reduction in vacuolation. Biochemically a reduction (62% in the cerebrum and 40% in the cerebellum) was demonstrated in IDU-PC-005 as compared to the untreated MPS I dogs.

The preclinical pharmacodynamic studies submitted in the IND suggest that enzyme replacement with rhIDU is effective in reducing the GAG accumulation in several soft tissues including the liver, spleen, lymph nodes, adrenal glands, small intestine, gall bladder and joint synovium in the both canine and feline MPS I disease models as determined by histology. However, the clinical significance of these histologic findings was not directly addressed in the studies and the lack of histologically significant changes in GAG accumulation in cartilage and the central nervous system suggest that the treatment may not prove effective against the orthopedic and cognitive manifestations of the clinical disease. The studies do not provide evidence that at the doses administered rhIDU is able to penetrate and accumulate to effective concentrations in cartilage and

central nervous system tissues, however the studies do not preclude the possibility that a higher dose or more frequent dosing regimen may produce enzyme concentrations sufficient to produce histologically significant changes in GAG accumulation in tissues that appear resistant to the short-term treatment. It should be noted that in addition to biochemical effects (increases in a-L-iduronidase activity levels in most tissues, decreases in GAG levels in tissues and urine, and histopathological evidence of tissue and organ improvement) demonstrated in the short term animal models long-term treatment of MPS I dogs (up to 74 weeks) with rhIDU resulted in improvement of clinical symptoms.

Subsequent formal toxicity studies in rats, dogs and monkeys further support the relative safety of rhIDU treatment. Of note there were only limited pharmacokinetic studies conducted and this topic should be addressed in more detail in post-marketing commitments. However the studies do suggest that the doses used in the clinical trial are sufficient to saturate a high affinity cellular receptor recognizing IDU. Acute toxicity studies were limited to a 15 day single dose IV study in the ----------rats and a single day repeat dose, dose escalation study in the ------. Neither study revealed toxicities. The primary toxicology study was a 26-week study in ---------- monkeys with 26 weekly IV bolus doses with a two-week recovery period. The highest dose used was 10 fold the dose used in the clinical trials. All monkeys in the high dose group had slightly elevated total leukocyte, lymphocytes, monocytes, and eosinophil counts that returned to normal levels after treatment (except eosinophils in males and monocytes in females). There was a single animal (152259) with a hypersensitivity reaction as described earlier in this section of the review. Approximately half of the monkeys developed anti-rhIDU antibodies. Neither the frequency nor the titer of the antibody was dose-related. The effect of the antibodies on pharmacokinetics was not well studied. Antibody production and pharmacokinetics can be further examined in phase IV studies. An eight-week repeat dose study was conducted to beagles to determine if changes in formulation effected the severity of infusion reactions. Although all the formulations tested exhibited infusion reactions after the third weekly dose the formulation incorporating Tween-80 produced the less severe reaction. Reproduction studies conducted in ----------rats failed to demonstrate deleterious effects on fertility, reproduction or development. In summary, the preclinical data adequately support use of the product, Aldurazyme^{TM}, for the indication specified by the sponsor.

_____ M. David Green Ph.D., Branch Chief

_____ Richard D. McFarland Ph.D., M.D., Medical Officer

Key Words; Hurler syndrome; Hurler-Scheie syndrome; Scheie Syndrome; Aldurazyme™; laronidase; mucopolysaccharidosis I; MPS I; α-L-iduronidase enzyme deficiency; lysosomes; lysosomal storage disease; antibodies; reproductive/developmental toxicity; non-human primate.

cc:

OTRR/DCTDA/CPT/MGreen

References

Clarke LA, Russell CS, Pownall S, Warrington CL, Borowski A, Dimmick JE, Toone J, Jirik FR. Murine mucopolysaccharidosis type I: Targeted disruption of the murine alpha-L-iduronidase gene. Hum Molec Genet 6:503-511, 1997.

Haskins ME, Desnick RJ, DiFerrante N, Jezyk PF, Patterson DF. Beta glucuronidase deficiency in a dog: A model of mucopolysaccharidosis VII. Pediatr Res 18:980-984, 1984

Haskins ME, Giger U. Lysosomal storage diseases in clinical biochemistry of domestic animals (Kaneko JJ, Harvey JW, Bruss ML, Eds.). New York: Academic Press, pp.741-761, 1997.

He X, Li C-M, Simonaro CM, Wan Q, Haskins ME, Desnick RJ, Schuchman EH. Identification and characterization of the molecular lesion causing mucopolysaccharidosis type I in cats. Molec Genet Metab 67:106-112, 1999.

Kakkis ED, McEntee MF, Schmidtchen A, Neufeld EF, Ward DA, Gompf RE et al. Long-Term and High-Dose Trials of Enzyme Replacement Therapy in the Canine Model of Mucopolysaccharidosis I. Biochem Mol Med 1996; 58(2):156-167.

Kakkis ED, Schuchman E, He X, Wan Q, Kania S, Wiemelt S et al. Enzyme Replacement Therapy in Feline Mucopolysaccharidosis I. Mol Genet Metab 2001; 72(3):199-208.

Cellular/Molecular

Clearance of α-Synuclein Oligomeric Intermediates via the Lysosomal Degradation Pathway

He-Jin Lee, Farnaz Khoshaghideh, Smita Patel, and Seung-Jae Lee
The Parkinson's Institute, Sunnyvale, California 94089

Cytoplasmic deposition of α-synuclein aggregates is a common pathological feature of many neurodegenerative diseases. Strong evidence for the causative role of α-synuclein in these disorders is provided by genetic linkage between this gene and familial Parkinson's disease and by neurodegeneration in transgenic animals that overexpress this protein. In particular, it has been hypothesized that the accumulation of nonfibrillar oligomers of α-synuclein, which serve as intermediates for fibrillar inclusion body formation, causes neurodegeneration. However, little is known about how cells handle potentially toxic protein aggregates. Here we demonstrate that cells are capable of clearing preformed α-synuclein aggregates via the lysosomal degradation pathway. Consequently, blocking this pathway causes the accumulation of the aggregates in non-neuronal cells, differentiated neuroblastoma cells, and primary cortical neurons. This aggregate clearance occurs in an aggregation stage-specific manner; oligomeric intermediates are susceptible to clearance, whereas mature fibrillar inclusion bodies are not. Neutralization of the acidic compartments leads to the accumulation of α-synuclein aggregates and exacerbates α-synuclein toxicity in postmitotic neuronal cells, suggesting that the accumulation of oligomeric intermediates may be an important event leading to α-synuclein-mediated cell death. These results suggest that enhancing lysosomal function may be a potential therapeutic strategy to halt or even prevent the pathogenesis of Parkinson's disease and other Lewy body diseases.

Key words: α-synuclein; protein aggregation; lysosome; Parkinson's disease; Lewy body; neurodegeneration

Introduction

Deposition of filamentous α-synuclein (α-syn) in the neuronal or glial cytoplasm is a common pathological feature of many neurological diseases, such as Parkinson's disease (PD), dementia with Lewy bodies, multiple system atrophy, and neuronal degeneration with brain iron accumulation type 1 (Trojanowski et al., 1998; Goedert, 2001). All of the genetic variations in human α-syn gene that are causative to the early-onset familial parkinsonism increase the probability to form aggregates (Polymeropoulos et al., 1997; Kruger et al., 1998; Conway et al., 2000; Singleton et al., 2003). Animal models developed in mice and flies have shown that overexpression of α-syn in neurons can cause neuronal loss, along with α-syn aggregation that leads to the formation of both filamentous and granular aggregates (Feany and Bender, 2000; Masliah et al., 2000; Giasson et al., 2002; M. Lee et al., 2002), further supporting the hypothesis that abnormal accumulation of α-syn aggregates may play a critical role in the pathogenesis of neurodegenerative diseases.

Biochemical studies have shown that α-syn can form amyloid-like fibrils with a cross-β-sheet conformation (Serpell et al., 2000). These fibrils have a similar morphology to that found in Lewy bodies (LBs) (Spillantini et al., 1998), suggesting that the mechanism of cell-free fibrillation may reflect the actual pathogenic process *in vivo*. Fibrillation of α-syn initiates with the dimerization of partially folded monomers (Uversky et al., 2001; Krishnan et al., 2003), followed by the formation of β-sheet-rich nonfibrillar oligomeric intermediates, also known as protofibrils, with several distinct morphologies (Volles and Lansbury, 2003). More recently, we have developed a cell culture model in which overexpression of human α-syn leads to the formation of LB-like inclusion bodies that consist mainly of fibrillar aggregates. In these cells we have demonstrated that nonfibrillar spherical oligomers act as intermediates in the formation of fibrillar inclusion bodies and that the oligomer-to-fibril transition requires microtubule-dependent deposition of oligomers in the pericentriolar region (Lee and Lee, 2002). The mechanism underlying the deleterious effects of α-syn aggregates remains to be elucidated. Several studies have suggested that the oligomeric intermediates are the cause of cellular dysfunction and cell death (Gosavi et al., 2002; Kayed et al., 2003; Volles and Lansbury, 2003), whereas others have pointed to the direct role of fibrillar inclusion bodies in neurodegeneration (for review, see Giasson and Lee, 2003).

The extent of aggregate accumulation likely is determined by a dynamic equilibrium between the production and clearance of aggregates, and this process may, in turn, be critical for cell viability. In contrast to the rapid progress in our understanding of the α-syn aggregation process and its regulation, little is known about the breakdown of preformed aggregates. Previously, we have shown that α-syn aggregation can be promoted by treating

Received Aug. 14, 2003; revised Dec. 23, 2003; accepted Dec. 23, 2003.

This work was supported by the Michael J. Fox Foundation for Parkinson's Research and the Abramson Family Foundation. Monoclonal antibody for LAMP 2 (H4B4) developed by Drs. August and Hildreth was obtained from the Developmental Studies Hybridoma Bank, developed under the auspices of the National Institute of Child Health and Human Development and maintained by The University of Iowa, Department of Biological Sciences, Iowa City, IA. We thank Stephen Lee and Amy Manning-Bog for critically reading this manuscript and Nafisa Ghori for technical assistance with EM.

Correspondence should be addressed to Seung-Jae Lee, The Parkinson's Institute, 1170 Morse Avenue, Sunnyvale, CA 94089. E-mail: slee@thepi.org.

DOI:10.1523/JNEUROSCI.3809-03.2004

 0270-6474/04/241888-09$15.00/0

cells with the common pesticide rotenone and that the cells can clear the preformed aggregates after removal of the rotenone from the culture medium (H.-J. Lee et al., 2002b). Here we further characterize this aggregate-clearing activity in both nonneuronal and neuronal cells and show that only the oligomeric intermediates, but not the fibrillar inclusion bodies, can be cleared via the lysosomal degradation pathway. We also demonstrate that lysosomal failure leads to the accumulation of α-syn aggregates and concomitant cell death, suggesting that the accumulation of oligomeric intermediates might be the key component of α-syn-mediated cytotoxicity.

Materials and Methods

Materials. Bafilomycin A1 (Baf), E64, cathepsin I, β-lactone, and epoxomicin (Epox) were obtained from Calbiochem (San Diego, CA). Retinoic acid (RA), 3-methyladenine (3-MA), and protease inhibitor mixture were purchased from Sigma (St. Louis, MO). Monoclonal antibodies for α-syn, LB509 and Syn-1, were purchased from Zymed Laboratories (South San Francisco, CA) and BD Biosciences (San Diego, CA), respectively. Polyclonal anti-α-syn 7071 was a gift from Dr. Lansbury (Harvard Medical School, Boston, MA). Monoclonal antibody for lysosome-associated membrane protein 2 (LAMP 2; H4B4) was obtained from the Developmental Studies Hybridoma Bank (Iowa City, IA). All of the fluorescently labeled secondary antibodies were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA). Gold-conjugated anti-mouse IgG antibody was obtained from Ted Pella (Redding, CA).

Adenoviral vectors. Construction of adeno/α-syn was described previously (H.-J. Lee et al., 2002b). For the construction of adenoviral vectors that contain myc and his-tagged α-syn variants, we first inserted PCR-amplified α-syn cDNAs into the *Eco*RV–*Xba*I site of pCDNA3.1-MycHis (Invitrogen, Carlsbad, CA). Coding regions for α-syn variants with the tag then were amplified via PCR and inserted into the transfer vector, pDNR-CMV (BD Biosciences). The sequences of entire coding regions of wild-type, A53T, and A30P variants were confirmed. Recombinant adenoviral DNAs were generated *in vitro* by the Adeno-X Expression System 2 (BD Biosciences).

Cell culture and α-syn expression. Cells were maintained as described previously (Lee and Lee, 2002). For expression of α-syn in COS-7 cells, 80% confluent cells on 100 mm tissue culture dishes were infected with adenoviral vector containing human α-syn cDNA (adeno/α-syn) (H.-J. Lee et al., 2002b) at a multiplicity of infection (m.o.i.) of 50. After 90 min of incubation fresh medium was added, and the cells were incubated at 37°C until the next day. Cells then were split to ~40% confluency and maintained for the indicated period. For induction of α-syn aggregation, rotenone was added at a concentration of 100 nM on the same day that the infected cells were split. For washout of rotenone, rotenone-containing medium was replaced with fresh rotenone-free medium. SH-SY5Y cells were split to ~10% confluency and the next day were induced to differentiate by the addition of 50 μM all-*trans* RA. The cells were fed with fresh medium containing RA every other day. On day 5 of differentiation the cells were infected with adeno/α-syn at a m.o.i. of 100. Unlike COS-7 cells, infected SH-SY5Y cells were maintained without splitting.

Primary cortical neurons were obtained from embryonic day 16 (E16) embryos of pregnant Sprague Dawley rats (Price and Brewer, 2001). Briefly, embryonic brains were placed in a sterile culture dish containing HBSS (*Invitrogen*), and cerebral cortices were dissected and collected in a centrifuge tube. The cortical tissues were incubated with the papain solution (HBSS containing 10 U/ml papain, 0.2 mg/ml cysteine, 0.5 mM EDTA, 1 mM $CaCl_2$, and 0.003N NaOH), followed by incubation with the trypsin inhibitor solution (MEM with Earl's salts containing 5% fetal bovine serum, 2.5 mg/ml bovine serum albumin, and 2.5 mg/ml trypsin inhibitor). Then the tissues were dissociated mechanically by pipetting in the culture medium and were centrifuged briefly to collect the cells. Cells were resuspended, plated on either poly-D-lysine/laminin-coated plastic dishes or poly-D-lysine/Matrigel-coated (1:100; BD Biosciences) glass coverslips, and cultured in Neurobasal medium (Invitrogen) with 2% B-27 supplement (Invitrogen) and 0.5 mM Glutamax-1 (Invitrogen). Culture medium was changed every 3 d.

Cell extraction and fractionation. For simple detergent extraction the cells were washed twice with ice-cold PBS before the addition of cold extraction buffer (PBS containing 1% Triton X-100 and protease inhibitor mixture). Cells were scraped, resuspended by pipetting, and put on ice for 10 min. Then the extracts were centrifuged at 16,000 × *g* for 10 min to separate Triton-soluble (supernatant) and Triton-insoluble (pellet) fractions. The amount of protein was measured with a BCA protein assay kit (Pierce, Rockford, IL). For separation of α-syn oligomers and mature inclusion bodies the procedure was performed as described previously (Lee and Lee, 2002). Briefly, the cells were washed with PBS before the addition of buffer T [containing (in mM): 20 Tris, pH 7.4, 25 KCl, 5 $MgCl_2$ plus 0.25 M sucrose, 1% Triton X-100, protease inhibitor mixture]. After buffer T was removed gently from dishes, the remaining Triton-insoluble material was collected in buffer N (0.1 M Na_2CO_3, pH 11.5, protease inhibitor mixture) and centrifuged at 80 × *g* for 10 min. The pellet containing mature inclusion bodies was dissolved in 1× Laemmli sample buffer (SB), and oligomers in the supernatant were centrifuged further at 16,000 × *g* for 10 min and dissolved in 1× SB.

Western blotting. Western blot analysis was performed according to the procedure described previously (Lee and Lee, 2002).

Immunofluorescence. The cell staining and confocal microscopy were performed as described previously (Lee and Lee, 2002).

Electron microscopy. COS-7 cells were infected with adeno/α-syn at a m.o.i. of 100 and incubated for 4 d. The sections were prepared and incubated with LB509 antibody, followed by incubation with 10 nm gold-conjugated goat anti-mouse IgG antibody as described in Gosavi et al. (2002).

Cell viability assay. SH-SY5Y cells were split in 96-well plates at a density of 1×10^5/well and differentiated for 5 d with 50 μM all-*trans* RA. Differentiated SH-SY5Y cells were infected with either adeno/α-syn or empty control vector at a m.o.i. of 100. The medium was changed the next day, and the reagents were treated on day 2 after infection. Cell viability was quantified via the MTS reduction assay (Promega, Madison, WI), or Luminescence ATP detection assay (PerkinElmer Life Sciences, Boston, MA), according to the manufacturers' instructions. For trypan blue exclusion assay the cells were trypsinized, resuspended in medium, and mixed with trypan blue dye solution (Sigma) before being counted with a hemacytometer. All assays were performed three times or more.

Results

In our previous study we have shown the progressive maturation of α-syn aggregates from small spherical oligomers to large fibrillar inclusion bodies in a tissue culture model (Lee and Lee, 2002). This process can be promoted by addition of the known mitochondrial inhibitor rotenone, and the preformed aggregates can be cleared with removal of the rotenone (H.-J. Lee et al., 2002b). In the present study we have investigated the susceptibility of different aggregate species to this aggregate-clearing activity, the mechanism of the clearance, and the effect of this mechanism on cell viability. COS-7 cells expressing human α-syn were treated with rotenone for 48, 56, 64, and 72 hr, followed by washout and incubation in rotenone-free medium for 24 or 48 hr. The duration of rotenone treatment correlated positively with the level of aggregates (Fig. 1*A*). These aggregates disappeared with removal of the rotenone, but the efficiency of aggregate clearance decreased with longer periods of rotenone treatment. Densitometric analysis of α-syn aggregates showed that the level of aggregates induced for 48 and 56 hr dropped by ~90% after 2 d of washout, but those induced for 72 hr dropped by only 45% after the same washout period (Fig. 1*A*, bottom panel). One interpretation of this finding is that α-syn aggregates acquire resistance to the clearance mechanism as they evolve into more mature forms.

To test this hypothesis, we analyzed separately the susceptibility of oligomeric intermediates and mature fibrillar inclusion

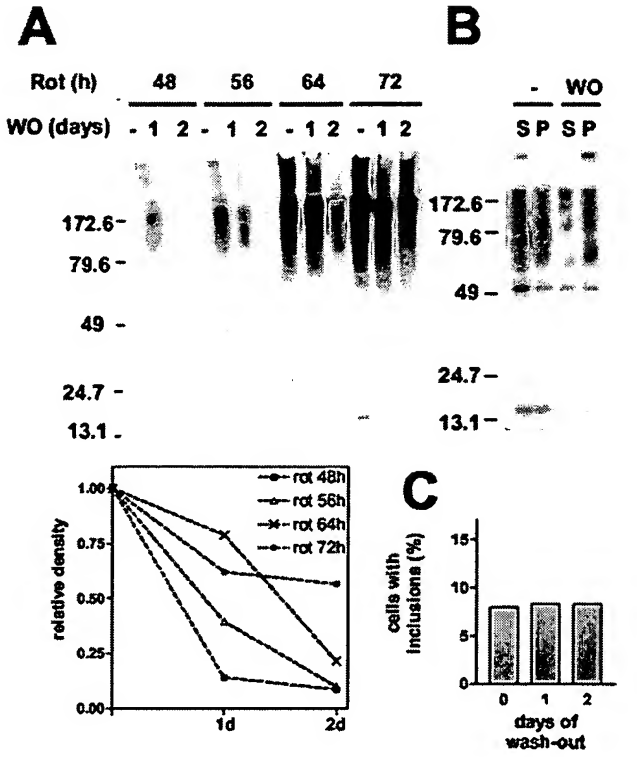


Figure 1. Clearance of α-synuclein oligomeric intermediates. COS-7 cells expressing α-syn were treated with 100 nM rotenone for the indicated times and then incubated in fresh medium without rotenone for 1 or 2 d. *A*, Cells were extracted with PBS/1% Triton X-100, and the Triton-insoluble fractions were analyzed by Western blotting. The bottom panel shows densitometric analysis of the Western data. The relative density is obtained by calculating the percentage of remaining aggregates after the rotenone washout. *B*, Oligomeric intermediates (S) and mature fibrillar inclusion bodies (P) were separated before (–) and 1 d after (WO) the rotenone washout and were analyzed by Western blotting. *C*, Quantitation of the number of mature inclusion bodies. Before and after the rotenone washout the cells were fixed and labeled for α-syn by immunofluorescence. Cells with mature juxtanuclear inclusion bodies were counted in six different randomly selected areas, and the number of these cells was divided by the number of nuclei to obtain the percentage.

bodies to the clearance mechanism after the rotenone washout. These aggregates can be separated and quantified via a simple centrifugation procedure; complete separation of these species was confirmed previously with electron microscopy (EM) (Lee and Lee, 2002). At 1 d after removal of the rotenone the level of oligomeric intermediates was significantly lower, whereas that of mature inclusion bodies remained the same (Fig. 1*B*). The resistance of large inclusions to clearance was confirmed in a microscopic analysis, which showed little change in the number of mature inclusion bodies during the rotenone washout (Fig. 1*C*). These results suggest that preformed α-syn aggregates can be cleared from the cytoplasm and that early-stage oligomeric intermediates are more susceptible to clearance than mature fibrillar inclusion bodies.

To elucidate the mechanism underlying α-syn aggregate clearance, we investigated the effects of various inhibitors of proteasomes and lysosomes. COS-7 cells expressing α-syn were treated with rotenone for 56 hr; then the medium was replaced with fresh medium lacking rotenone but with various chemicals and incubated for 20 hr. Treatment of the cells with Baf, a specific inhibitor of vacuolar H^+-ATPase that disrupts the pH gradient in acidic organelles including lysosomes (Bowman et al., 1988), abolished the aggregate-clearing activity in a dose-dependent manner, causing a net increase in the level of α-syn aggregates

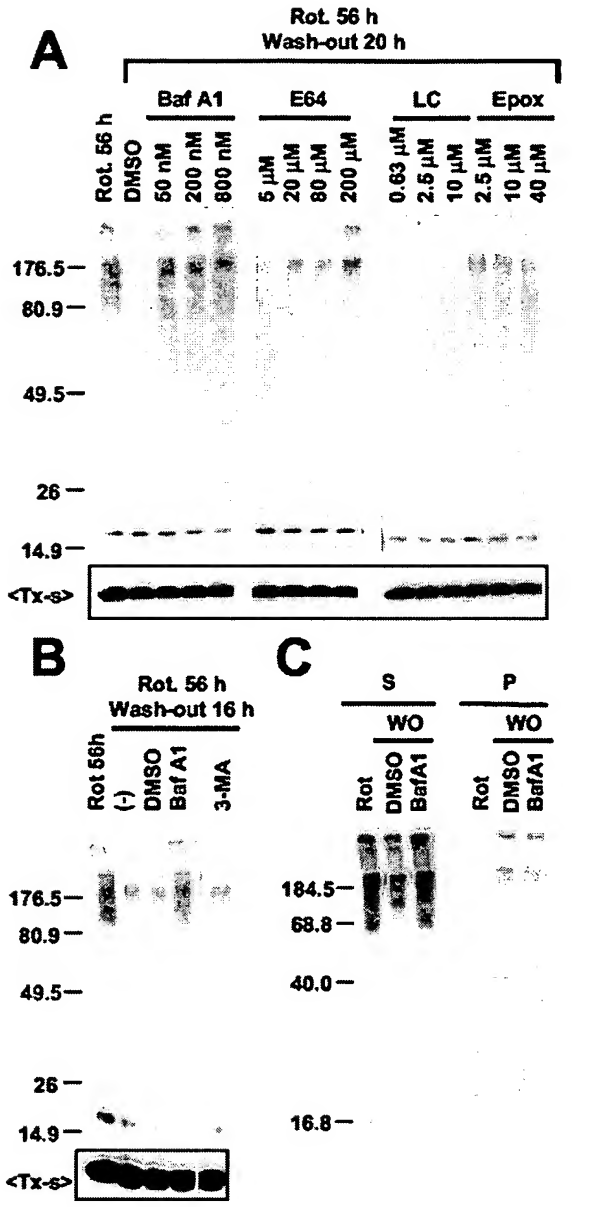


Figure 2. The mechanism of α-syn aggregate clearance. COS-7 cells expressing α-syn were treated with 100 nM rotenone for 56 hr, followed by rotenone washout. *A*, Lysosomal and proteasomal inhibitors were added at the indicated concentrations during the rotenone washout. *B*, During the rotenone washout COS-7 cells were treated with Baf (200 nM) or 3-MA (10 mM); (–) indicates no-treatment control. DMSO was treated as a vehicle control for Baf. *C*, After 16 hr of the rotenone washout (WO) in the presence of DMSO or Baf, oligomeric aggregates (S) and mature inclusion bodies (P) were separated and analyzed by Western blotting. In *A* and *B*, the top panels show Triton-insoluble aggregates, and the bottom panels show monomers in the Triton-soluble fractions (TX-s).

(Fig. 2*A*). In support of these data, E64, an irreversible cysteine–protease inhibitor that affects a subset of lysosomal proteases, also showed a dose-dependent inhibition of aggregate-clearing activity, although to a lesser extent. These results suggest that the lysosomal degradation pathway may be important for the clearance of α-syn aggregates. On the other hand, although the proteasome inhibitors β-lactone (LC) and Epox partially inhibited the aggregate clearance, their effects plateaued at a level that was much less than that of Baf (Fig. 2*A*). This result suggests that proteasomes contribute only partially to aggregate clearance and that the majority of aggregates might not be susceptible to

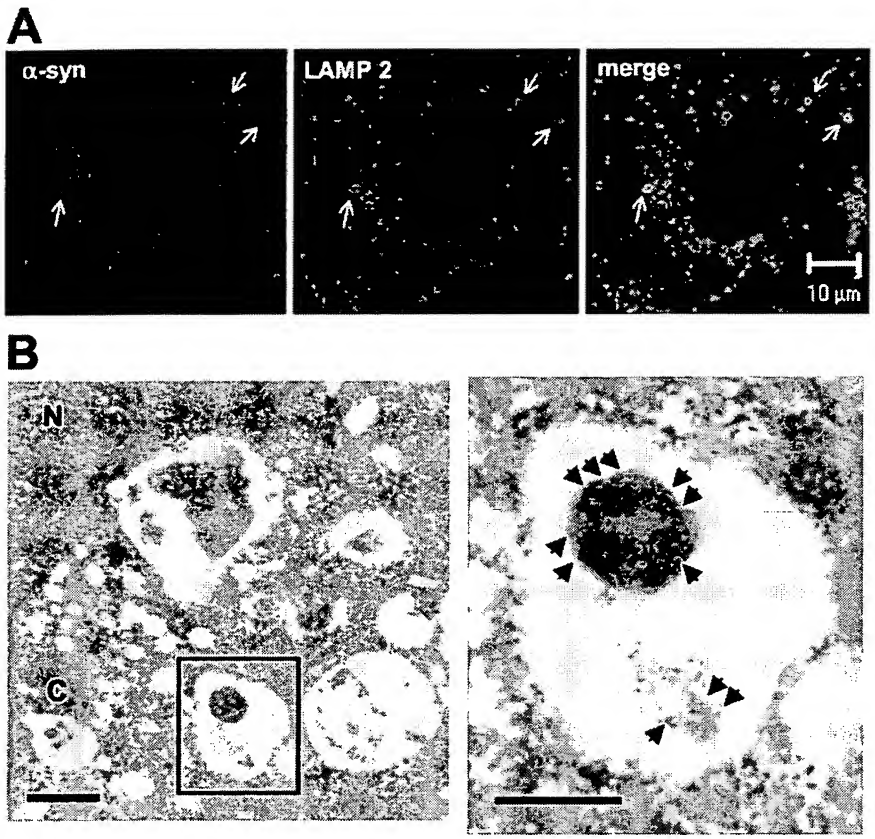


Figure 3. Localization of α-syn aggregates in the lysosomes. *A*, COS-7 cells expressing α-syn were treated with 100 nM rotenone for 56 hr, followed by incubation in rotenone-free medium. Cells were fixed and labeled with antibodies for α-syn (red) and LAMP 2 (green). Arrows indicate α-syn aggregates captured within LAMP 2-positive compartments. Nuclei were stained with Hoechst 33258 (blue). *B*, EM analysis of COS-7 cells with α-syn aggregates. α-Syn aggregates are visualized by 10 nm gold-conjugated secondary antibody. Boxed area is magnified on the right. Scale bars: left, 0.4 μm; right, 0.2 μm. Arrowheads indicate immunogold particles that label spherical and amorphous aggregates in a vacuolar structure. The amorphous aggregate may represent ongoing degradation. N, Nucleus; C, centrosome.

proteasome-mediated degradation. Because Baf also is known to inhibit macroautophagy by inhibiting autophagosome–lysosome fusion (Kim and Klionsky, 2000), we examined the effect of 3-MA, an inhibitor of the early sequestration stage of macroautophagy (Seglen and Gordon, 1982). Unlike Baf, 3-MA showed little effect on the clearance of α-syn aggregates (Fig. 2*B*). A macroautophagy stimulator, rapamycin (Blommaart et al., 1995), did not show a significant effect on aggregate clearance either (data not shown). It is unlikely that the effects of these reagents on the levels of α-syn aggregates are attributable to the changes in monomer levels, because none of these reagents showed significant effect on monomer levels within the time scale of the experiment (Fig. 2*A*,*B*; TX-s). Using the fractionation procedure that separates small oligomers and mature inclusion bodies, we confirmed that oligomeric intermediates are the targets of Baf-sensitive clearance (Fig. 2*C*).

The involvement of the lysosomal pathway in α-syn aggregate clearance was demonstrated further by microscopic examination. COS-7 cells expressing α-syn were treated with rotenone for 56 hr, followed by incubation in rotenone-free medium for 20 hr. Immunofluorescence labeling of these cells showed that α-syn aggregates occasionally are surrounded by LAMP 2, a protein that is localized in the limiting membranes of the late endosomes and lysosomes (Fig. 3*A*, arrows). Immuno-EM study also showed the localization of α-syn-positive aggregates in vacuolar structures (Fig. 3*B*). These results further strengthen the conclusion that spherical α-syn oligomers are cleared from the cytoplasm via the lysosomal degradation pathway.

To determine whether postmitotic neuronal cells also can clear α-syn aggregates, we characterized α-syn aggregation in SH-SY5Y human neuroblastoma cells. With RA treatment these cells differentiate into postmitotic neuron-like cells with extended neurites and neuronal marker proteins. At different stages of the differentiation the cells were infected with adeno/α-syn and incubated for 3 d (Fig. 4*A*). The cells were lysed in the presence of 1% Triton X-100, and the Triton-soluble and Triton-insoluble fractions were subjected to Western blotting by the use of anti-α-synuclein antibody. The Triton-soluble and Triton-insoluble fractions are defined in this study as the supernatant and the pellet of 16,000 × *g* centrifugation, respectively. Naive SH-SY5Y cells overexpressing α-synuclein did not produce a detectable level of aggregates, whereas the same level of expression induced aggregation in differentiated SH-SY5Y cells; the level of aggregation increased with a longer period of differentiation (Fig. 4*B*).

Aggregation of α-syn in differentiated SH-SY5Y has some characteristics that are distinct from those in COS-7 cells. Unlike COS-7 cells in which most aggregates are found in the Triton-insoluble fraction, we found that differentiated SH-SY5Y cells produced Triton-soluble and Triton-insoluble aggregates equally well (Fig. 4*B*). The Triton-soluble fraction was centrifuged sequentially at increasing centrifugal forces for 1 hr, and we found that the Triton-soluble aggregates remained in the supernatant even at 200,000 × *g* (Fig. 4*C*). This indicates that differentiated SH-SY5Y cells produce stable small oligomers in addition to larger oligomers that sediment at 16,000 × *g*. Rotenone had relatively little effect on the level of α-syn aggregates in differentiated SH-SY5Y cells, resulting in less than a twofold increase (data not shown), indicating subtle cell type-specific differences in the regulation of α-syn aggregation. However, microscopic characterization demonstrated that differentiated SH-SY5Y cells produce both spherical oligomers and mature inclusion bodies that have structural and compositional properties similar to those produced in COS-7 cells (Supplementary Fig. 1; available at www.jneurosci.org). Thus despite some differences the overall mechanism of α-syn aggregation in differentiated SH-SY5Y cells appears to be similar to that in COS-7 cells.

To determine whether α-syn aggregates also can be removed via the lysosomal pathway in differentiated SH-SY5Y cells, we treated cells overexpressing α-syn with lysosomal inhibitors. Treatment with Baf led to the accumulation of both the Triton-soluble and Triton-insoluble aggregates in a dose- and time-dependent manner, whereas little change was observed in the levels of monomer (Fig. 5*A*,*B*). On the other hand, Baf treatment did not cause the accumulation of α-syn aggregates in undifferentiated SH-SY5Y cells (Fig. 5*C*), in which aggregate formation

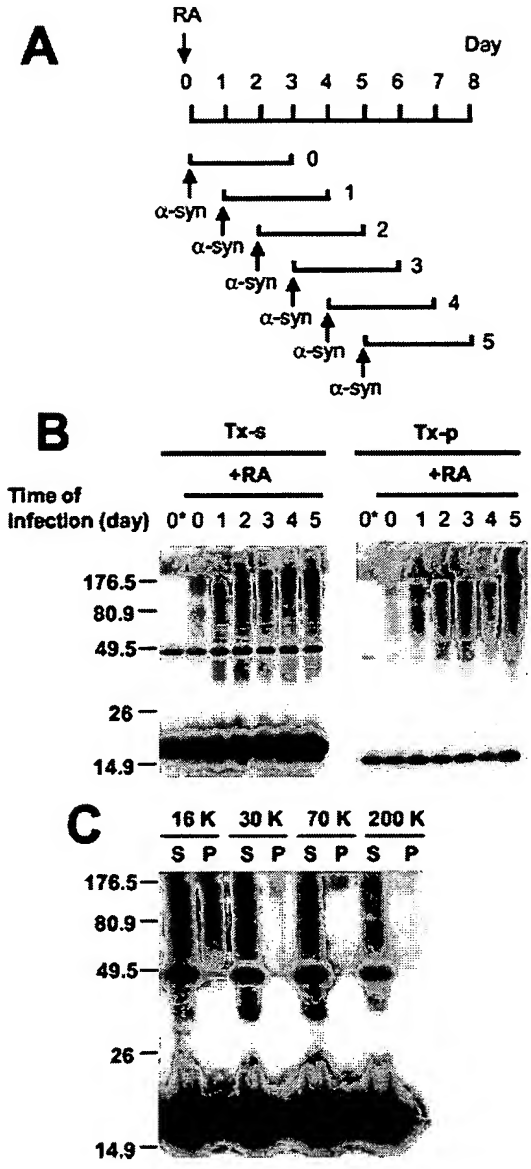


Figure 4. α-Synuclein aggregation in differentiated human neuroblastoma cells. *A*, Schematic presentation of differentiation and α-synuclein expression. α-Synuclein is expressed for 3 d in cells at different stages of differentiation. *B*, Western analysis of α-synuclein aggregation. The first lane (0*) of each panel shows the naive SH-SY5Y cells infected and processed in the same way. Note that the level of α-synuclein aggregation increases with a longer period of differentiation in both Triton-soluble (TX-s) and Triton-insoluble (TX-p) fractions. *C*, Sedimentation analysis of α-syn aggregates. Detergent extract of differentiated SH-SY5Y cells overexpressing α-syn was subjected to a sequential differential centrifugation. Cells were treated with 20 nM Baf before the extraction to enrich the aggregates (see Fig. 5). Numbers at the top indicate the centrifugal forces in gravity (*g*). S and P indicate the supernatant and the pellet of each centrifugation, respectively.

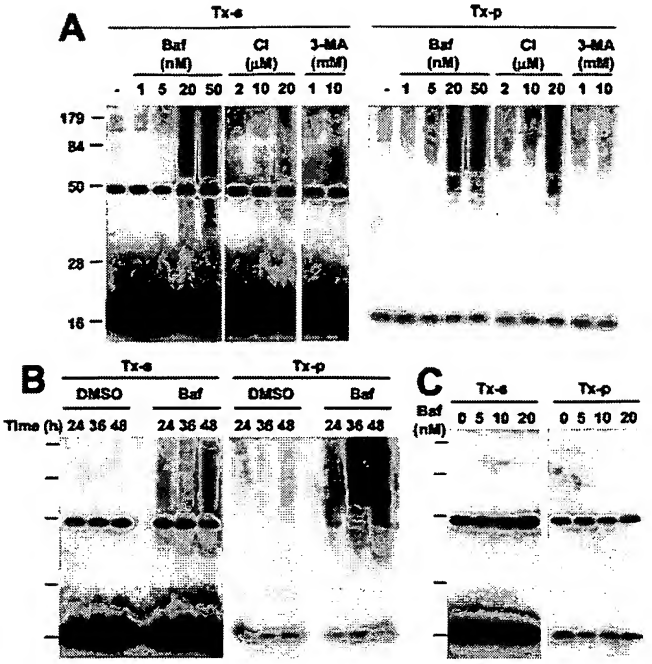


Figure 5. Lysosomal inhibitors stabilize α-syn aggregates in differentiated SH-SY5Y cells. *A*, Differentiated SH-SY5Y cells overexpressing α-syn were treated with Baf, cathepsin inhibitor I, or 3-MA for 24 hr at the indicated concentrations. Triton-soluble (TX-s) and Triton-insoluble (TX-p) fractions were obtained from cell extracts and analyzed by Western blotting. *B*, Progressive accumulation of aggregates in differentiated SH-SY5Y after treatment with 20 nM Baf. *C*, Baf treatment does not lead to aggregate accumulation in undifferentiated SH-SY5Y cells.

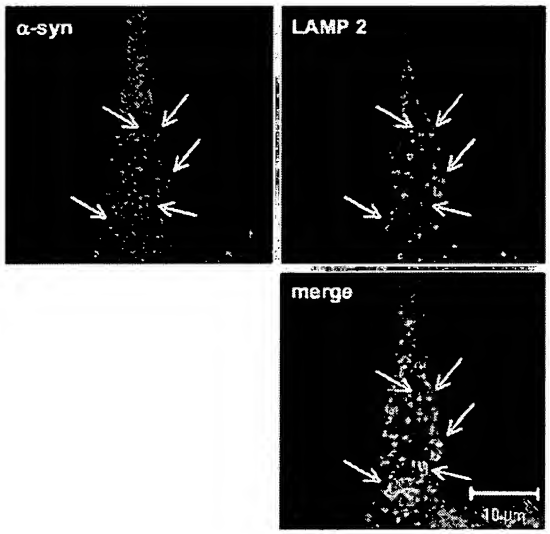


Figure 6. Overlap between α-syn punctate pattern and LAMP 2 in differentiated SH-SY5Y cells. Cells overexpressing α-syn were treated with 4 μM cathepsin inhibitor I for 24 hr and labeled with antibodies for α-syn (red) and LAMP 2 (green). Arrows indicate granular α-syn stains that overlap with LAMP 2-positive compartments. Nuclei were stained with Hoechst 33258 (blue).

was not detected (Fig. 4). This suggests that Baf affects the clearance of preformed aggregates rather than their *de novo* formation; if production were altered, Baf treatment would have increased the aggregate levels in undifferentiated cells. Cathepsin I, an inhibitor of lysosomal cathepsin proteases, also increased the level of α-syn aggregates, confirming the role of the lysosomal pathway. In contrast, neither aggregate nor monomer levels changed after the treatment with macroautophagy inhibitor 3-MA. These results suggest that the non-macroautophagic lysosomal degradation pathway might be involved in α-syn aggregate clearance in postmitotic neuronal cells. This is supported further by the finding that oligomeric aggregates often colocalize with LAMP 2 in differentiated SH-SY5Y cells treated with a lysosomal protease inhibitor (Fig. 6).

We have shown previously that overexpression of α-syn in COS-7 cells leads to a reduction in cell viability and that cell death correlates with the formation of oligomeric intermediates

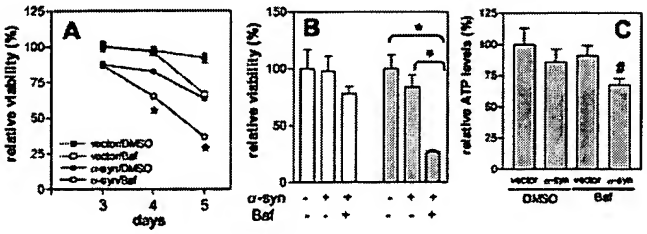


Figure 7. Lysosomal inhibition exacerbates α-syn-induced cytotoxicity. *A*, Differentiated SH-SY5Y cells were infected with empty control vector (dotted lines) or adeno/α-syn (solid lines) and were treated with DMSO (filled symbols) or 10 nM Baf (open symbols) on day 2 after infection. Cell viability was measured on days 3, 4, and 5 after infection, using the MTS reduction assay. To obtain relative viability, we used data from empty vector-infected cells on day 3 as a reference. *B*, Trypan blue exclusion assay. Cells were treated with 10 nM Baf on day 2 after infection, and live cell numbers were obtained on days 3 (white bars) and 4 (gray bars). *C*, ATP measurement. Cells were treated with 10 nM Baf, and ATP levels were measured on day 4. *A*, *B*, $n = 3$; *C*, $n = 5$. Error bars represent SEM. Statistical significance was assessed by one-way ANOVA and is indicated with $^{*}p < 0.01$ and $^{\#}p < 0.05$.

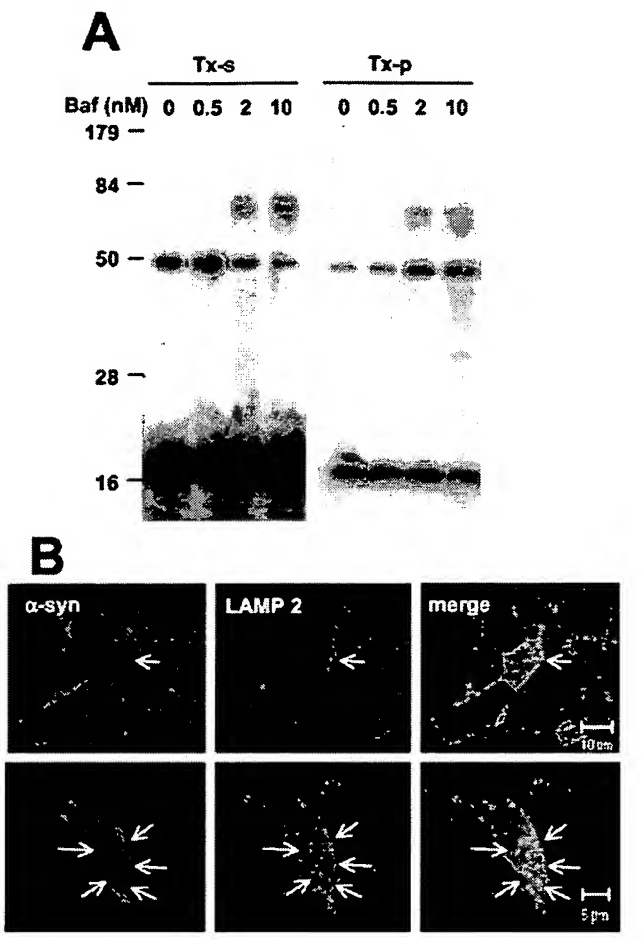


Figure 8. Accumulation of α-syn oligomers in rat cortical neurons. *A*, Neurons (DIV 11) were treated with Baf at the indicated concentrations for 24 hr. Endogenous α-syn in the Triton-soluble (TX-s) and Triton-insoluble (TX-p) fractions was analyzed by Western blotting. *B*, DIV 11 cortical neurons were treated with the mixture of serine, cysteine, and aspartic protease inhibitors for 2 hr (top images) and 4 hr (bottom images) and fluorescently labeled for α-syn (red) and LAMP 2 (green). Arrows indicate the colocalization between α-syn and LAMP 2. Nuclei were stained with Hoechst 33258 (blue).

(Gosavi et al., 2002). Differentiated SH-SY5Y cells overexpressing α-syn also showed reduced cell viability as compared with cells transduced with empty control vector, indicating that overproduction of α-syn has a toxic effect (Fig. 7*A*, filled square vs filled circle). To study the effect of Baf on α-syn-induced cell toxicity, we transduced the cells with either empty vector or adeno/α-syn and then treated them with Baf on day 2 after infection. Cell viability was measured by the MTS reduction assay. Treatment with Baf increased the toxic effect of α-syn even further, resulting in a greater rate of cell death as compared with DMSO-treated α-syn-expressing cells (Fig. 7*A*, filled vs open circle). The effect of Baf on α-syn-overexpressing cells became apparent on day 4, which is 2 d after the treatment, whereas Baf treatment of cells transduced with empty control vector did not cause significant cell death during the same period (Fig. 7*A*, open square). This suggests that Baf itself did not contribute directly to cell death until day 4, and that it did so by potentiating the toxic effects of α-syn. Enhancement of α-syn cytotoxicity in Baf-treated cells was confirmed by two additional assays: live cell counting (Fig. 7*B*) and ATP measurement (Fig. 7*C*). These results indicate that stabilization of α-syn oligomers by inhibition of the lysosomal pathway exacerbates α-syn toxicity.

Having demonstrated the effect of the lysosomal defect on the stabilization of α-syn aggregates in cells transiently overexpressing α-syn, we then asked whether the lysosomal defect has the same effect on endogenous neuronal α-syn. To investigate this, we studied the effect of Baf on α-syn aggregate levels in rat embryonic cortical neurons, which express high levels of endogenous α-syn. Neurons (11 d *in vitro*, DIV 11) were treated with increasing amounts of Baf, and high-molecular-weight α-syn aggregates were visualized by Western blotting. After Baf treatment the neurons accumulated a 62 kDa oligomeric α-syn in both Triton-soluble and Triton-insoluble fractions (Fig. 8*A*). Because the 62 kDa oligomer was identified in denaturing polyacrylamide gel, this oligomer could be a stable unit of much larger complex rather than an independent oligomer on its own. This finding confirms that lysosomal defect leads to the accumulation of an oligomeric form of α-syn generated from the endogenous protein in cortical neurons. Furthermore, when the neurons were treated with a mixture of protease inhibitors that block the activities of most of lysosomal proteolytic enzymes, the punctate pattern of α-syn immunoreactivity appeared in a time-dependent manner and overlapped with the LAMP 2-positive compartments (Fig. 8*B*), suggesting that the oligomers that fail to be degraded accumulate in the lysosomes.

Discussion

In the present study we confirmed that cells, including those of neuronal origin, are capable of clearing preformed α-syn aggregates. More importantly, the following findings point to the conclusion that only the oligomeric intermediates, but not the mature fibrillar inclusion bodies, are susceptible to the aggregate-clearing mechanism. First, α-syn aggregates become resistant to breakdown as the aggregates advance to more mature states (i.e., fibrillar inclusion bodies). Second, aggregate breakdown is observed only in the oligomer-enriched fraction, not in the mature inclusion body fraction. Third, the number of mature inclusion bodies does not decrease during the period in which oligomers undergo an extensive breakdown. Inhibition of this clearance mechanism causes rapid accumulation of aggregates in both differentiated human neuroblastoma cells and rat embryonic cortical neurons. These results suggest that aggregate clearance is a major determinant of the cellular α-syn aggregate load, thus demonstrating that the extent of accumulation of α-syn aggre-

gates is determined by a dynamic equilibrium between production and removal of the aggregates.

Our study shows that the lysosomal degradation pathway is mostly responsible for the clearance of α-syn oligomers. This is supported by the findings that neutralization of acidic compartments and inhibition of lysosomal proteases block the oligomer clearance and that α-syn oligomers are present in LAMP 2-positive vacuolar structures. Although the proteasomal pathway does appear to contribute to aggregate clearance, it does so to a much lesser extent than the lysosomal pathway. This is consistent with the finding that oligomeric aggregates are mostly ubiquitin-negative and rarely colocalize with proteasomes (Lee and Lee, 2002) (see also Supplementary Fig. 1). Whether there are different populations of aggregates that are cleared distinctly by either the lysosomal or the proteasomal pathway is an important issue that needs to be addressed.

Although the mechanism of α-syn degradation is far from clearly understood, both the proteasome and lysosome systems appear to play a role in normal metabolism of this protein (Bennett et al., 1999; Paxinou et al., 2001; Tofaris et al., 2001; Liu et al., 2003; Webb et al., 2003). Because protein aggregation is sensitive to the expression level of protein, the accumulation of α-syn aggregates in response to various inhibitors of these degradation systems actually might be the consequence of simply elevating the cytoplasmic α-syn concentration. However, in our study the accumulation of α-syn oligomers occurs rapidly, within a matter of hours, whereas the estimated half-life of α-syn is as long as several days (Okochi et al., 2000; Paxinou et al., 2001). In fact, within the time scale of our experiments we did not detect significant changes in the monomer levels. Thus it is unlikely that changes in the monomer levels play a significant role in the observed effects of the lysosomal inhibitors on the aggregate accumulation.

One crucial question is how the aggregates are delivered to the lysosomes for destruction. Although little is known specifically about the delivery of protein aggregates to the lysosomes, a process called autophagy has been considered a major pathway for targeting various cytoplasmic substrates destined for degradation in lysosomes. Autophagy has been linked with some programmed cell death (Xue et al., 1999; Inbal et al., 2002) and is hyperactivated both in a mouse model of neurodegeneration (Yue et al., 2002) and in human neurodegenerative diseases, such as PD (Anglade et al., 1997) and Alzheimer's disease (Nixon et al., 2000). In culture the expression of mutant huntingtin in striatal neurons (Kegel et al., 2000; Petersen et al., 2001) and exposure of dopaminergic neurons to methamphetamine (Larsen et al., 2002) caused autophagy-associated degeneration. Furthermore, overexpression of the A53T mutant form of α-syn stimulated autophagic cell death in PC12 cells (Stefanis et al., 2001). There are distinct autophagic mechanisms: macroautophagy, microautophagy, and chaperone-mediated autophagy. Delivery of bulk cytoplasmic "garbage" to lysosomes is mediated by macroautophagy. This process is initiated by enclosing obsolete organelles and proteins with double membrane structures called autophagosomes, which then are fused with the lysosomes to form autophagic vacuoles (Kim and Klionsky, 2000). Microautophagy, on the other hand, occurs when lysosomes invaginate their own membranes, resulting in direct uptake of parts of the cytoplasm (Dunn, 1994). Another mechanism called chaperone-mediated autophagy involves direct import of cytosolic proteins into the lysosomes (Chiang et al., 1989).

To assess the role of autophagy in α-syn aggregate clearance, we investigated the effects of macroautophagy inhibition and stimulation and found that neither inhibition nor stimulation showed a significant effect. Consistent with this finding, visualization of autophagic vesicles with a fluorescent dye, monodansylcadaverine (Biederbick et al., 1995), showed no colocalization between α-syn aggregates and these vesicles (data not shown). Although macroautophagy does not appear to play an important role in the breakdown of α-syn aggregates, other autophagic mechanisms might be involved and thus are worth further investigation. Alternatively, it is also possible that the endosomal pathway, which is another mechanism of substrate delivery for lysosomal degradation, may play a role in the clearance of α-syn aggregates. Interestingly, we have shown recently that α-syn aggregation can initiate and develop in membranes (H.-J. Lee et al., 2002a). These membrane-associated aggregates thus may be targeted to the lysosomes via the endosomal pathway.

We have identified several distinct oligomeric species both in COS-7 cells (Lee and Lee, 2002) and in differentiated SH-SY5Y cells (S.-J. Lee, unpublished observations); their sizes range widely from dimer to multimers. COS-7 and differentiated SH-SY5Y cells show distinct patterns of size distribution at steady state: COS-7 cells mostly have multimers large enough to sediment at 16,000 × *g*, whereas more than one-half of the SH-SY5Y aggregates are found in the supernatant of a 200,000 × *g* spin. The multimers found in the 16,000 × *g* pellet are spherically shaped, with sizes of 15–60 nm in diameter (Lee and Lee, 2002), much larger than the spherical protofibrils (2–8 nm) produced from purified recombinant α-syn by Lansbury and colleagues (Ding et al., 2002). Some smaller multimers, on the other hand, show sedimentation properties similar to the *in vitro* protofibrils (Y. S. Kim and S.-J. Lee, unpublished observations). Except for the relative sizes, there is no structural information available at the moment for distinguishing these aggregate species between themselves and from the *in vitro* protofibrils. Therefore, we call all of the nonfibrillar aggregates collectively "oligomers" in this report. In cells overexpressing α-syn, these nonfibrillar oligomers appear before the formation of the juxtanuclear inclusion bodies that contain α-syn fibrils, and both the fibrillation and the inclusion body formation are blocked by disrupting the transport of these oligomers (Lee and Lee, 2002). These results support the conclusion that some of these oligomers, if not all, are the precursors of the inclusion bodies and the intermediates of the cellular fibrillation process.

In the differentiated SH-SY5Y model we show that the accumulation of oligomeric intermediates correlates with α-syn-mediated cytotoxicity. Some studies have raised the possibility that α-syn oligomers act directly as proteotoxins (Volles et al., 2001; Kayed et al., 2003). Alternatively, it is also plausible that the accumulation of these oligomers might overburden the cellular machinery that is involved in the transport and clearance of these aggregates. This abnormally high burden not only might disturb normal cell physiology but also might render cells highly sensitive to additional stresses. However, it needs to be noted that the accumulation of oligomers would lead to the formation of mature inclusion bodies, and there is a view that inclusion bodies are participating directly in cellular dysfunction and demise (Giasson and Lee, 2003). Both in cells and in test tubes the α-syn fibrillation process appears to involve multiple rounds of conversion from one intermediate form to more advanced forms. How this process occurs in a cellular context and how the cells respond to these aggregates on their accumulation are critical questions that remain to be addressed. Solving these questions should advance our understanding of the mechanism that underlies α-syn cytotoxicity and, eventually, of the pathogenesis of α-synucleinopathies.

Lysosomal processing capacity appears to diminish progres-

sively over the lifespan of an animal (Brunk and Brun, 1972; Nakamura et al., 1989), and lysosomal malfunction has been associated with age-related neurodegenerative disorders (Nixon et al., 2000; Bahr and Bendiske, 2002). The link between lysosomal dysfunction and neurodegeneration has been strengthened further by the finding that the inhibition of lysosomal enzymes leads to some of the features of age-related neurodegenerative diseases, such as protein deposition, synaptic loss, and neuronal demise (Takauchi and Miyoshi, 1989; Okada et al., 1994; Bendiske et al., 2002; Felbor et al., 2002). The results presented in the current study link lysosomal disturbance to the accumulation of α-syn oligomers and α-syn-mediated cell death, implicating the importance of the lysosomal pathway in the cellular response to α-syn aggregation. We propose that the lysosomal pathway is a central protective mechanism against α-syn oligomer-mediated toxicity and that stimulation of this pathway may be an effective approach to the prevention and treatment of PD and other related disorders.

References

Anglade P, Vyas S, Javoy-Agid F, Herrero MT, Michel PP, Marquez J, Mouatt-Prigent A, Ruberg M, Hirsch EC, Agid Y (1997) Apoptosis and autophagy in nigral neurons of patients with Parkinson's disease. Histol Histopathol 12:25–31.

Bahr BA, Bendiske J (2002) The neuropathogenic contributions of lysosomal dysfunction. J Neurochem 83:481–489.

Bendiske J, Caba E, Brown QB, Bahr BA (2002) Intracellular deposition, microtubule destabilization, and transport failure: an "early" pathogenic cascade leading to synaptic decline. J Neuropathol Exp Neurol 61:640–650.

Bennett MC, Bishop JF, Leng Y, Chock PB, Chase TN, Mouradian MM (1999) Degradation of α-synuclein by proteasome. J Biol Chem 274:33855–33858.

Biederbick A, Kern HF, Elsasser HP (1995) Monodansylcadaverine (MDC) is a specific *in vivo* marker for autophagic vacuoles. Eur J Cell Biol 66:3–14.

Blommaart EF, Luiken JJ, Blommaart PJ, van Woerkom GM, Meijer AJ (1995) Phosphorylation of ribosomal protein S6 is inhibitory for autophagy in isolated rat hepatocytes. J Biol Chem 270:2320–2326.

Bowman EJ, Siebers A, Altendorf K (1988) Bafilomycins: a class of inhibitors of membrane ATPases from microorganisms, animal cells, and plant cells. Proc Natl Acad Sci USA 85:7972–7976.

Brunk U, Brun A (1972) The effect of aging on lysosomal permeability in nerve cells of the central nervous system. An enzyme histochemical study in rat. Histochemie 30:315–324.

Chiang HL, Terlecky SR, Plant CP, Dice JF (1989) A role for a 70-kilodalton heat shock protein in lysosomal degradation of intracellular proteins. Science 246:382–385.

Conway KA, Lee S-J, Rochet JC, Ding TT, Williamson RE, Lansbury Jr PT (2000) Acceleration of oligomerization, not fibrillization, is a shared property of both α-synuclein mutations linked to early-onset Parkinson's disease: implications for pathogenesis and therapy. Proc Natl Acad Sci USA 97:571–576.

Ding TT, Lee S-J, Rochet JC, Lansbury Jr PT (2002) Annular alpha-synuclein protofibrils are produced when spherical protofibrils are incubated in solution or bound to brain-derived membranes. Biochemistry 41:10209–10217.

Dunn Jr W (1994) Autophagy and related mechanisms of lysosome-mediated protein degradation. Trends Cell Biol 4:139–143.

Feany MB, Bender WW (2000) A *Drosophila* model of Parkinson's disease. Nature 404:394–398.

Felbor U, Kessler B, Mothes W, Goebel HH, Ploegh HL, Bronson RT, Olsen BR (2002) Neuronal loss and brain atrophy in mice lacking cathepsins B and L. Proc Natl Acad Sci USA 99:7883–7888.

Giasson BI, Lee VM (2003) Are ubiquitination pathways central to Parkinson's disease? Cell 114:1–8.

Giasson BI, Duda JE, Quinn SM, Zhang B, Trojanowski JQ, Lee VM-Y (2002) Neuronal alpha-synucleinopathy with severe movement disorder in mice expressing A53T human alpha-synuclein. Neuron 34:521–533.

Goedert M (2001) Alpha-synuclein and neurodegenerative diseases. Nat Rev Neurosci 2:492–501.

Gosavi N, Lee H-J, Lee JS, Patel S, Lee S-J (2002) Golgi fragmentation occurs in the cells with prefibrillar α-synuclein aggregates and precedes the formation of fibrillar inclusion. J Biol Chem 277:48984–48992.

Inbal B, Bialik S, Sabanay I, Shani G, Kimchi A (2002) DAP kinase and DRP-1 mediate membrane blebbing and the formation of autophagic vesicles during programmed cell death. J Cell Biol 157:455–468.

Kayed R, Head E, Thompson JL, McIntire TM, Milton SC, Cotman CW, Glabe CG (2003) Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. Science 300:486–489.

Kegel KB, Kim M, Sapp E, McIntyre C, Castano JG, Aronin N, DiFiglia M (2000) Huntingtin expression stimulates endosomal–lysosomal activity, endosome tubulation, and autophagy. J Neurosci 20:7268–7278.

Kim J, Klionsky DJ (2000) Autophagy, cytoplasm-to-vacuole targeting pathway, and pexophagy in yeast and mammalian cells. Annu Rev Biochem 69:303–342.

Krishnan S, Chi EY, Wood SJ, Kendrick BS, Li C, Garzon-Rodriguez W, Wypych J, Randolph TW, Narhi LO, Biere AL, Citron M, Carpenter JF (2003) Oxidative dimer formation is the critical rate-limiting step for Parkinson's disease alpha-synuclein fibrillogenesis. Biochemistry 42:829–837.

Kruger R, Kuhn W, Muller T, Woitalla D, Graeber M, Kosel S, Przuntek H, Epplen JT, Schols L, Riess O (1998) Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. Nat Genet 18:106–108.

Larsen KE, Fon EA, Hastings TG, Edwards RH, Sulzer D (2002) Methamphetamine-induced degeneration of dopaminergic neurons involves autophagy and upregulation of dopamine synthesis. J Neurosci 22:8951–8960.

Lee H-J, Lee S-J (2002) Characterization of cytoplasmic α-synuclein aggregates. Fibril formation is tightly linked to the inclusion-forming process in cells. J Biol Chem 277:48976–48983.

Lee H-J, Choi C, Lee S-J (2002a) Membrane-bound α-synuclein has a high aggregation propensity and the ability to seed the aggregation of the cytosolic form. J Biol Chem 277:671–678.

Lee H-J, Shin SY, Choi C, Lee YH, Lee S-J (2002b) Formation and removal of α-synuclein aggregates in cells exposed to mitochondrial inhibitors. J Biol Chem 277:5411–5417.

Lee MK, Stirling W, Xu Y, Xu X, Qui D, Mandir AS, Dawson TM, Copeland NG, Jenkins NA, Price DL (2002) Human α-synuclein harboring familial Parkinson's disease-linked Ala-53→Thr mutation causes neurodegenerative disease with α-synuclein aggregation in transgenic mice. Proc Natl Acad Sci USA 99:8968–8973.

Liu CW, Corboy MJ, DeMartino GN, Thomas PJ (2003) Endoproteolytic activity of the proteasome. Science 299:408–411.

Masliah E, Rockenstein E, Veinbergs I, Mallory M, Hashimoto M, Takeda A, Sagara Y, Sisk A, Mucke L (2000) Dopaminergic loss and inclusion body formation in alpha-synuclein mice: implications for neurodegenerative disorders. Science 287:1265–1269.

Nakamura Y, Takeda M, Suzuki H, Morita H, Tada K, Hariguchi S, Nishimura T (1989) Lysosome instability in aged rat brain. Neurosci Lett 97:215–220.

Nixon RA, Cataldo AM, Mathews PM (2000) The endosomal–lysosomal system of neurons in Alzheimer's disease pathogenesis: a review. Neurochem Res 25:1161–1172.

Okada M, Miyake T, Kitamura T, Kawasaki K, Mizushima Y (1994) Anti-ubiquitin immunoreactivity associates with pyramidal cell death induced by intraventricular infusion of leupeptin in rat hippocampus. Neurosci Res 19:59–66.

Okochi M, Walter J, Koyama A, Nakajo S, Baba M, Iwatsubo T, Meijer L, Kahle PJ, Haass C (2000) Constitutive phosphorylation of the Parkinson's disease associated α-synuclein. J Biol Chem 275:390–397.

Paxinou E, Chen Q, Weisse M, Giasson BI, Norris EH, Rueter SM, Trojanowski JQ, Lee VM, Ischiropoulos H (2001) Induction of α-synuclein aggregation by intracellular nitrative insult. J Neurosci 21:8053–8061.

Petersen A, Larsen KE, Behr GG, Romero N, Przedborski S, Brundin P, Sulzer D (2001) Expanded CAG repeats in exon 1 of the Huntington's disease gene stimulate dopamine-mediated striatal neuron autophagy and degeneration. Hum Mol Genet 10:1243–1254.

Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papapetropoulos T, Johnson WG, Lazzarini AM, Duvoisin

RC, Di Iorio G, Golbe LI, Nussbaum RL (1997) Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 276:2045–2047.

Price PJ, Brewer GJ (2001) Serum-free media for neural cell cultures: adult and embryonic. In: Protocols for neural cell culture, 3rd Ed (Fedoroff S, Richardson A, eds), pp 255–264. Totowa, NJ: Humana.

Seglen PO, Gordon PB (1982) 3-Methyladenine: specific inhibitor of autophagic/lysosomal protein degradation in isolated rat hepatocytes. Proc Natl Acad Sci USA 79:1889–1892.

Serpell LC, Berriman J, Jakes R, Goedert M, Crowther RA (2000) Fiber diffraction of synthetic α-synuclein filaments shows amyloid-like cross-β conformation. Proc Natl Acad Sci USA 97:4897–4902.

Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, Kachergus J, Hulihan M, Peuralinna T, Dutra A, Nussbaum R, Lincoln S, Crawley A, Hanson M, Maraganore D, Adler C, Cookson MR, Muenter M, Baptista M, Miller D, Blancato J, Hardy J, Gwinn-Hardy K (2003) α-Synuclein locus triplication causes Parkinson's disease. Science 302:841.

Spillantini MG, Crowther RA, Jakes R, Hasegawa M, Goedert M (1998) α-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with Lewy bodies. Proc Natl Acad Sci USA 95:6469–6473.

Stefanis L, Larsen KE, Rideout HJ, Sulzer D, Greene LA (2001) Expression of A53T mutant but not wild-type α-synuclein in PC12 cells induces alterations of the ubiquitin-dependent degradation system, loss of dopamine release, and autophagic cell death. J Neurosci 21:9549–9560.

Takauchi S, Miyoshi K (1989) Degeneration of neuronal processes in rats induced by a protease inhibitor, leupeptin. Acta Neuropathol (Berl) 78:380–387.

Tofaris GK, Layfield R, Spillantini MG (2001) Alpha-synuclein metabolism and aggregation is linked to ubiquitin-independent degradation by the proteasome. FEBS Lett 509:22–26.

Trojanowski JQ, Goedert M, Iwatsubo T, Lee VM (1998) Fatal attractions: abnormal protein aggregation and neuron death in Parkinson's disease and Lewy body dementia. Cell Death Differ 5:832–837.

Uversky VN, Lee H-J, Li J, Fink AL, Lee S-J (2001) Stabilization of partially folded conformation during α-synuclein oligomerization in both purified and cytosolic preparations. J Biol Chem 276:43495–43498.

Volles MJ, Lansbury Jr PT (2003) Zeroing in on the pathogenic form of alpha-synuclein and its mechanism of neurotoxicity in Parkinson's disease. Biochemistry 42:7871–7878.

Volles MJ, Lee S-J, Rochet JC, Shtilerman MD, Ding TT, Kessler JC, Lansbury Jr PT (2001) Vesicle permeabilization by protofibrillar alpha-synuclein: implications for the pathogenesis and treatment of Parkinson's disease. Biochemistry 40:7812–7819.

Webb JL, Ravikumar B, Atkins J, Skepper JN, Rubinsztein DC (2003) α-Synuclein is degraded by both autophagy and the proteasome. J Biol Chem 278:25009–25013.

Xue L, Fletcher GC, Tolkovsky AM (1999) Autophagy is activated by apoptotic signaling in sympathetic neurons: an alternative mechanism of death execution. Mol Cell Neurosci 14:180–198.

Yue Z, Horton A, Bravin M, DeJager PL, Selimi F, Heintz N (2002) A novel protein complex linking the delta 2 glutamate receptor and autophagy: implications for neurodegeneration in *lurcher* mice. Neuron 35:921–933.

FDA Talk Paper

T04-09
April 21, 2004

Media Inquiries: 301-827-6242
Consumer Inquiries: 888-INFO-FDA

***** CLARIFICATION *****
Paragraph three revised 04/26/2004.

FDA Approves Apokyn for the Acute Treatment of Episodes of Immobility in Parkinson's Patients

FDA has approved Apokyn (apomorphine) as an injectable drug for treating Parkinson's patients during episodes of "hypomobility," so-called "off periods" in which the patient becomes immobile or unable to perform activities of daily living. Apokyn was given priority review because injectable apomorphine is the first therapy approved to treat these episodes acutely (during the episode). Apokyn was also designated as an orphan product.

An estimated 1.5 million Americans have Parkinson's disease, which results in tremors, rigidity, postural instability, slowness, and difficulty moving.

Within 3 to 5 years of treatment with standard Parkinson's drug treatments, many patients experience episodes of hypomobility (e.g., inability to rise from a chair, to speak, or to walk). The episodes can occur toward the end of a dosing interval with standard background medications (so-called "end-of-dose wearing off") or at unpredictable times (spontaneous "on/off"). Approximately 10 percent of Parkinson's patients who are unresponsive to standard medications may benefit from Apokyn.

Apokyn was designated an orphan drug in 1991 to treat the ten percent, or about 112,000 Parkinson's patients who progress to stage four and experience the severe on/off motor fluctuations unresponsive to other therapies. Orphan drugs are drugs that treat a rare disease or condition which affects fewer than 200,000 patients in the U.S. After receiving FDA approval, orphan drugs are eligible for seven years of exclusive marketing.

The effectiveness of Apokyn in the acute symptomatic treatment of both types of recurring episodes of hypomobility or "off" episodes associated with advanced Parkinson's disease was established in three randomized, controlled trials. On average, patients participating in these trials had had Parkinson's disease for 11.3 years and were being treated with L-dopa and at least one other agent, usually an oral dopamine agonist.

Apokyn must be taken with an antiemetic drug because, when taken alone, it causes severe nausea and vomiting. It must not be taken with one class of very effective anti-nausea drugs, the 5HT3 antagonists (ondansetron and similar drugs), because the combination of Apokyn (apomorphine) and these drugs can lead to very low blood pressure and loss of consciousness.

Apokyn is intended for subcutaneous injection only. Other oral drugs, taken chronically, are also used to help decrease the amount of time Parkinson's patients spend in the "off" state.

DGNews

FDA Approves Namenda (Memant HCl) for Treatment of Moderate to S Alzheimer's Disease

NEW YORK, NY -- October 17, 2003 -- Forest Laboratorie (NYSE: FRX) announced today that Namenda™ (memanti first of a new class of drugs for Alzheimer's disease, was ap the U.S. Food and Drug Administration (FDA) for the treat moderate to severe Alzheimer's disease. Forest expects Nam available to physicians, patients, and pharmacies in January Namenda is the first NMDA receptor antagonist to be appro Alzheimer's disease and is also the only therapy approved f treatment of moderate to severe Alzheimer's disease.

"The approval of Namenda offers an important new therape for patients suffering from moderate to severe Alzheimer's d Howard Solomon, Chairman and Chief Executive Officer o Laboratories. "Previously patients with moderate disease ha class of options; now they have an additional therapy availa patients who had progressed beyond the moderate stage of A disease had no approved therapeutic option at all. We believ will provide a meaningful benefit to millions of Americans from Alzheimer's disease, whether as a patient, caregiver, o member."

Namenda will be available in pharmacies in January 2004. I

Note:

These press releases and statements were accurate, in all material respects, at the time of their issuance. However, Johnson & Johnson and the operating companies assume no obligation to update, correct or otherwise modify any of this material. We recommend that you view the most recent press releases and statements in order to receive the most current information made available by Johnson & Johnson.

REMINYL®, New Treatment For Alzheimer's Disease, Receives FDA Approval

REMINYL® Derived from the Daffodil

Titusville, NJ (February 28, 2001) – REMINYL® (galantamine hydrobromide) – a new treatment for mild to moderate Alzheimer's disease derived from the bulbs of daffodils – was approved today by the U.S. Food and Drug Administration (FDA).

Data from four placebo-controlled, double-blind clinical trials involving more than 2,650 patients show that REMINYL® can have a beneficial effect on patients' daily function and ability to think. To be available by prescription in May, REMINYL® was developed by the Janssen Research Foundation under a co-development and licensing agreement with the UK-based Shire Pharmaceuticals Group plc.

An estimated four million Americans have Alzheimer's disease – a progressive loss of cognitive function (thinking, remembering and reasoning) so severe that it interferes with an individual's ability to function. That number is expected to grow to 14 million by the middle of the next ce disorder is the third-most-expensive illness in the United States, behind on disease and cancer.

"Alzheimer's disease patients progressively deteriorate," says Gary Small, I of the Center on Aging and professor of psychiatry and biobehavioral scien University of California in Los Angeles. "But the studies show that REMINYL benefit many individuals with the disease. In studies lasting up to six mont patients' symptoms initially improved or stabilized, and even when they be decline, they remained better than those who were treated with placebo."

In studies ranging from 12 to 26 weeks, the effectiveness of REMINYL® wa using two primary tools. Patients' abilities related to memory, orientation, and language were assessed using the cognitive portion of the Alzheimer's Assessment Scale (ADAS-cog). Across all studies, the results consistently demonstrated that more patients taking REMINYL® showed significant imp their cognitive performance than those receiving placebo (inactive medicati

The second primary measure of effectiveness was the Clinician's Interview-Impression of Change plus Caregiver Information (CIBIC-plus), which prov

Archive

- Ortho-McNeil Pl Inc., Receives S TOPAMAX® (December 12,
- Study Suggests CONSTA™, Nev For Schizophrer Further Relieve Stable Patients (December 10,
- ALZA Corporati 48 Filing (December 9, 2
- Holiday Break I Creates Challen Children, Careg (December 9, 2
- Treating Schizo Patients With R CONSTA™ May Hospitalization Cost of Care (December 9, 2
- FDA Approves I for Treatment c Mania (December 5, 2
- More News

FAQs

Description of Medical Areas

About the FDA Approved Listings

Drugs Approved by the FDA

Drug Name: Exelon (rivastigmine tartrate)

Back

The following information is obtained from various newswires, published medical journal articles, and medical conference presentations.

Company: Novartis
Approval Status: Approved April 2000
Treatment for: Indicated for the treatment of mild to moderate dementia of the Alzheimer's type

---

General Information

Exelon has been approved in oral solution *and* capsule form for the treatment of mild to moderate Alzheimer's disease. This drug belongs to the class of drugs called cholinesterase inhibitors. Cholinesterase breaks down acetylcholine, a neurotransmitter which assists in human memory and cognition processes. By inhibiting cholinesterase, more acetylcholine available to the patient for memory and cognitive functioning. This is effective in treatment of Alzheimer's disease, since acetylcholine is at significantly lower levels in Alzheimer's patients than in normally functioning people.

Exelon has been shown to improve patients' performance in the three major domains of assessment of Alzheimer's: global functioning (such as activities of daily living), behavior, and cognition.

Another long term advantage to the drug is that it could treat symptoms early on in the deterioration process. Delaying the onset of the disease by five years in patients could save up to $50 billion in U.S. healthcare costs annually (half of the current annual cost).

Currently, 70 countries have approved Exelon for marketing.

Alzheimer's Disease is a neurodegenerative disease affecting up to 4 million adults in the U.S. and 10 million worldwide. Memory loss and other cognitive and behavior deteriorations are symptoms of the disease. As there is no current cure, Alzheimer's Disease is fatal.

Clinical Results

The safety and efficacy of Exelon was investigated in two placebo-controlled

FAQs

Description of Medical Areas

About the FDA Approved Listings

Drugs Approved by the FDA

Drug Name: ARICEPT (donepezil hydrochloride)

The following information is obtained from various newswires, published medical journal articles, and medical conference presentations.

Company: Eisai
Approval Status: Approved December 1996
Treatment for: Alzheimer's Disease

General Information

Aricept has been approved for the symptomatic treatment of mild to moderate Alzheimer's disease. Aricept is effective in improving cognition and patient function in people with mild to moderate Alzheimer's Disease.

Clinical Results

Controlled clinical trials in over 900 subjects demonstrated that more than 80% of subjects taking Aricept either improved or exhibited no further demonstration in tests of cognition over the course of the studies. In an assessment of patient function, which includes general function, cognition, behavior and activities of daily living, clinicians rated approximately two times as many subjects on Aricept as improved in comparison to placebo after 24 weeks of treatment.

Mechanism of Action

Aricept is a new reversible inhibitor of the enzyme acetylcholinesterase. Acetylcholinesterase is an enzyme, which breaks down the neurotransmitter acetylcholine. Aricept may allow a greater concentration of acetylcholine in the brain, thereby improving cholinergic function. Acetylcholine, associated with memory and learning, is in short supply in subjects with Alzheimer's disease.

Drug listing last updated on June 29, 2004

PAC> 022660

CenterWatch Newly Approved Drug Therapies Listing

Comtan Receives FDA Approval For Manage Parkinson's Disease

EAST HANOVER, N.J. – October 20, 1999 – Comtan (entacapone), a new medication for Parkinson's Disea received marketing approval from the U.S. Food and D Administration (FDA).

Worldwide clinical studies and marketing experience ir approximately 30,000 patients have demonstrated that enhances the benefits of levodopa in patients with Par who experience end-of-dose "wearing off". Comtan is approved for marketing in more than 35 countries.

"Comtan is an important new treatment in the manage Parkinson's Disease because it can help improve mot performance and significantly increase the amount of patients by prolonging the benefits of levodopa/carbidc preparations, the mainstay therapy," said Dr. Lynn Kra President, Nervous System, Clinical Research and De Novartis Pharmaceuticals Corporation. 'On' time refers of relatively good function, marked by patients' ability t common but important daily activities such as walking, writing and dressing. 'Off' time is characterized by peri immobility and a time when patients with Parkinson's a incapable of independent movement.

Optimizing Standard Parkinson's Disease Treatment

Comtan belongs to a new therapeutic class called COI inhibitors that block the COMT enzyme, and thus, redu breakdown of levodopa before it reaches the brain. Th of a COMT inhibitor to the treatment regimen ensures use of the prescribed levodopa/carbidopa, resulting in more sustained availability of levodopa for brain entry extending the positive effect of each levodopa dose. C exposure to dopamine allows patients to function more independently and for longer periods of time between

Lipitor as a Treatment for Alzheimer's Disease

This study is no longer recruiting patients.

Sponsored by:	Institute for the Study of Aging Pfizer
Information provided by:	National Institute on Aging (NIA)

Purpose

The purpose of this study is to assess the clinical benefit of Lipitor, a cholesterol-lowering drug, in the treatment of Alzheimer's disease.

Condition	Treatment or Intervention	Phase
Alzheimer Disease	Drug: Atorvastatin calcium	Phase II

MedlinePlus related topics: Alzheimer's Caregivers; Alzheimer's Disease
Genetics Home Reference related topics: Alzheimer disease

Study Type: Interventional
Study Design: Treatment, Randomized, Double-Blind, Placebo Control, Efficacy Study

Official Title: Effect of the HMG-CoA Reductase Inhibitor Atorvastatin Calcium, Lipitor, in the Treatment of Alzheimer's Disease

Further Study Details:

Expected Total Enrollment: 98

Study start: October 2000; Study completion: April 2004
Last follow-up: August 2004

This study is a phase II, placebo controlled, double-blind, one year trial investigating the effect of HmG-CoA reductase inhibitor atorvastatin calcium in the treatment of persons with possible or probable Alzheimer's disease. Subjects may continue to take stable doses of Aricept and Exelon. Following enrollment, participants will make visits to the study center every three months for blood tests and neuropsychological testing.

Eligibility

Ages Eligible for Study: 50 Years and above, Genders Eligible for Study: Both

Criteria

APPENDIX C - THIS IS NOT PART OF BRIEF

Please place a copy of

9. A method for treating neurodegeneration in a subject, comprising:

administering to the subject a therapeutically effective amount of a lysosomal modulating compound, a physiologically acceptable salt of the lysosomal modulating compound or a combination thereof, wherein enzymatic capacity of lysosomes in the subject is enhanced.

APPENDIX D - THIS IS NOT PART OF BRIEF

Please place copies of Assignment, Assignment recordation here.

ASSIGNMENT

WHEREAS, I, Ben A. BAHR, residing at 214 Main Street, Hampton, Connecticut 06247, have invented new and useful improvements in

MATERIALS FOR LYSOSOME MODULATION AND METHODS OF USE THEREOF

for which I am making application for Letters Patent of the United States, which application was filed on 10/29/2001 under Application No. 10/056,666 ; (and I hereby authorize my attorneys authorized to prosecute said application to above, insert the filing date and serial number of said application as soon as it is known); and

WHEREAS, the UNIVERSITY OF CONNECTICUT, a nonprofit organization, having a place of business at 263 Farmington Avenue, Farmington, CT 06030, is desirous of acquiring the entire right, title and interest in and to said improvements and any Letters Patent which may be granted thereon;

NOW, THEREFORE, TO ALL WHOM IT MAY CONCERN, be it known that for and in consideration of the sum of One (1) Dollar to me in hand paid and other good and valuable consideration, receipt of which is hereby acknowledged, I, the said Ben A. BAHR, sell, assign and transfer to the University of Connecticut, its successors and assigns (hereinafter called "Assignee"), the entire right, title and interest in and to said improvements and in and to any Letters Patent which may be obtained thereon in the United States and in all countries foreign thereto, together with said application and all divisional, continuing, substitute, renewal, reissue, and other applications for Letters Patent which have been or may be filed on said improvements in the United States or any other country; the same to be held and enjoyed by the Assignee for its and their sole use and behoof; and I do hereby further assign to the Assignee the right to file applications for patent in all countries on said improvements and all rights of priority resulting from any application for Letters Patent filed on said improvements.

I hereby authorize and request the Commissioner of Patents and Trademarks to issue all Letters Patent of the United States on said improvements to the Assignee.

I further covenant and agree that when requested by the Assignee, and without further consideration, but at the cost and expense of the Assignee, I will, for any and all countries, execute and deliver all applications for patent on said improvements, execute all lawful oaths and other papers, supply to the Assignee all facts and evidence known to me relating to said improvements and the history and

development thereof, testify in all interferences, suits, and other legal proceedings, and generally do everything rightful which the Assignee shall consider desirable for aiding in securing, maintaining, and enforcing proper patent protection for said improvements and for vesting the title to said improvements in the Assignee.

I further covenant that I have the lawful right to assign the interest in said improvements in the manner and form as herein expressed and that the interests herein conveyed are free from prior assignment, grant, mortgage, license, or other encumbrance whatsoever.

IN WITNESS WHEREOF, I have hereunto set my hand and seal as of and for this 5 day of February, 2002.

Ben A. BAHR

State of CT)
) ss: Storrs
County of Tolland)

On this 5 day of February, 2002 before me personally appeared Ben A. BAHR, who acknowledged that he signed the within instrument and that he executed the same as his own free act and deed.

Deborah L. Gobert
Notary Public

Date Commission Expires:

DEBORAH L. GOBERT
NOTARY PUBLIC
MY COMMISSION EXPIRES FEB. 28, 2006